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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
C 1020 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/00367	23/01/1998	23/01/1997
International Patent Classification (IPC) or national classification and IPC		
A61K39/39		
Applicant		
WAGNER, Hermann et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/08/1998	16.07.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer: Halle, F Telephone No. (+49-89) 2399 8537

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/00367

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-28 as originally filed

Claims, No.:

1-9 as received on 02/07/1999 with letter of 02/07/1999

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/00367

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-9
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-9
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-9
	No:	Claims

2. Citations and explanations

see separate sheet

VIII. Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP98/00367

Point V

Having regard to the prior art, the subject-matter of claims 1-9 appears to be novel (Article 33(2) PCT) and to involve an inventive step (Article 33(3) PCT). The claimed subject-matter as presently defined with respect to the polynucleotide sequences comprising a binding site of a transcription factor does not appear to be anticipated by the prior art or to be obvious to the skilled person. Concerning the independent claim 4, it seems that the nature of the binding site could be more precisely defined, cf. Point VIII, below.

Point VIII

Independent claim 4 refers to the sequence of a binding site of a transcription factor. It seems that the instructions concerning the definition of said binding site of the transcription factor could be more precise in order to allow the skilled person to reduce them to practice without undue burden of experimentation (Article 6 and Rule 6.3(a) PCT).

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02. Juli 1999

PCT/EP98/00367
WAGNER, Hermann et al.
Our Ref.: C 1020 PCT

Claims

1. A pharmaceutical composition, comprising:
   (a) at least one polynucleotide, wherein the polynucleotide comprises the sequence of a binding site of a transcription factor and has a nucleic acid sequence of any one of SEQ ID NO: 8 (GATTGCCTGA CGTCAGAGAG), SEQ ID NO: 9 (GGAATGACGT TCCCTGTG), SEQ ID NO: 10 (AGCTATGACG TTCCAAGG), SEQ ID NO: 11 (GCTTGATGAC TCAGCCGGAA), SEQ ID NO: 12 (TCGATCGGGG CGGGGCGAGC), SEQ ID NO: 13 (TGCAGATTGC GCAATCTGCA), SEQ ID NO: 14 (AGCGGGGGCG AGCGGGGGCG), SEQ ID NO: 16 (GTCCATTTCC CGTAAATCTT), SEQ ID NO: 17 (TATGCATATT CCTGTAAGTG), SEQ ID NO: 19 (CTGATTTCCC CGAAATGATG), SEQ ID NO: 20 (AGATTTCTAG GAATTCAATC), SEQ ID NO: 21 (GTATTTCCCA GAAAAGGAAC), SEQ ID NO: 22 (AAGCGAAAAT GAAATTGACT), and SEQ ID NO: 23 (CAGGCATAAC GGTTCCGTAG);
   (b) at least one antigen; and
   (c) a pharmaceutically acceptable carrier and/or diluent.

2. The composition according to claim 1 characterized in that the polynucleotide comprises at least one phosphorothioate linkage.

3. Pharmaceutical composition according to any one of the preceding claims characterized in that the antigen (b) is selected from the group comprising peptides, polypeptides, proteins, polysaccharides, steroids and tumor cells.

AMENDED SHEET

4. Use of a polynucleotide, wherein the polynucleotide comprises the sequence of a binding site of a transcription factor, for the preparation of a pharmaceutical composition for inducing a cytolytic T lymphocyte response.

5. The use according to claim 4, wherein the polynucleotide has a nucleic acid sequence of any one of SEQ ID NO: 8 (GATTGCCTGA CGTCAGAGAG), SEQ ID NO: 9 (GGAATGACGT TCCCTGTG), SEQ ID NO: 10 (AGCTATGACG TTCCAAGG), SEQ ID NO: 12 (TCGATCGGGG CGGGGCGAGC), SEQ ID NO: 16 (GTCCATTTCC CGTAAATCTT), SEQ ID NO: 19 (CTGATTTCCC CGAAATGATG), SEQ ID NO: 21 (GTATTTCCCA GAAAAGGAAC), SEQ ID NO: 22 (AAGCGAAAAT GAAATTGACT), and SEQ ID NO: 23 (CAGGCATAAC GGTTCCGTAG).

6. Use of a polynucleotide, wherein the polynucleotide comprises the sequence of a binding site of a transcription factor and wherein the polynucleotide has a nucleic acid sequence of any one of SEQ ID NO: 8 (GATTGCCTGA CGTCAGAGAG), SEQ ID NO: 9 (GGAATGACGT TCCCTGTG), SEQ ID NO: 10 (AGCTATGACG TTCCAAGG), SEQ ID NO: 11 (GCTTGATGAC TCAGCCGGAA), SEQ ID NO: 12 (TCGATCGGGG CGGGGCGAGC), SEQ ID NO: 13 (TGCAGATTGC GCAATCTGCA), SEQ ID NO: 14 (AGCGGGGGCG AGCGGGGGCG), SEQ ID NO: 16 (GTCCATTTCC CGTAAATCTT), SEQ ID NO: 17 (TATGCATATT CCTGTAAGTG), SEQ ID NO: 19 (CTGATTTCCC CGAAATGATG), SEQ ID NO: 20 (AGATTTCTAG GAATTCAATC), SEQ ID NO: 21 (GTATTTCCCA GAAAAGGAAC), SEQ ID NO: 22 (AAGCGAAAAT GAAATTGACT), and SEQ ID NO: 23 (CAGGCATAAC GGTTCCGTAG), for the preparation of a pharmaceutical composition for modulating an immune response.

7. Use of a polynucleotide, wherein the polynucleotide comprises the sequence of a binding site of a transcription factor and wherein the polynucleotide has a nucleic acid sequence of any one of SEQ ID NO: 8 (GATTGCCTGA CGTCAGAGAG), SEQ ID NO: 16 (GTCCATTTCC CGTAAATCTT), and SEQ ID NO: 19 (CTGATTTCCC

AMENDED SHEET

CGAAATGATG), for the preparation of a pharmaceutical composition for inducing a Th2 immune response.

8. Use of a polynucleotide, wherein the polynucleotide comprises the sequence of a binding site of a transcription factor and wherein the polynucleotide has a nucleic acid sequence of any one of SEQ ID NO: 8 (GATTGCCTGA CGTCAGAGAG), SEQ ID NO: 12 (TCGATCGGGG CGGGGCGAGC), SEQ ID NO: 14 (AGCGGGGGCG AGCGGGGGCG), SEQ ID NO: 16 (GTCCATTTCC CGTAAATCTT), SEQ ID NO: 19 (CTGATTTCCC CGAAATGATG), SEQ ID NO: 22 (AAGCGAAAAT GAAATTGACT), and SEQ ID NO: 23 (CAGGCATAAC GGTTCCGTAG), for the preparation of a pharmaceutical composition for inducing a Th1 immune response.

9. Use according to any one of claims 6 to 9 wherein said pharmaceutical composition is a vaccine.

AMENDED SHEET

PARENT

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(54) Title: METHODS FOR DIAGNOSING AND TREATING BODY WEIGHT RELATED DISORDERS IN ANIMALS

(57) Abstract

A method for determining if an animal is at risk for a body weight related disorder is described. An animal is provided and an aspect of ICAM receptor or β_2 integrin metabolism or structure is evaluated in the animal. An abnormality in the aspect of ICAM receptor or β_2 integrin metabolism or structure is diagnosed of being at risk for a body weight related disorder. Also described are methods for detecting the presence of a disease affecting body weight, methods for evaluating an agent for use in modulating body weight, methods for treating a body weight related disorder, methods for monitoring a therapeutic treatment of a disease affecting body weight, and methods for increasing the fat content in the liver or milk of an animal. Pharmaceutical compositions are also provided.

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METHODS FOR DIAGNOSING AND TREATING BODY WEIGHT RELATED DISORDERS IN ANIMALS

This application claims the benefit of U.S. Provisional Application No. 60/035,896 filed January 24, 1997.

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant Nos. R01 HL53756 and R01 HL41002 awarded by the National Institutes of Health, National Heart, Lung and Blood Institute.

Field of the Invention

This invention relates generally to treatments, diagnoses, and drug screens for body weight related disorders in animals.

Background of the Invention

Body weight disorders, including obesity and severe weight loss, afflict a large number of people. Obesity is a condition in which there is an excess of body fat. It is often associated with other disease conditions including diabetes, high blood pressure and high cholesterol levels. Based on the Body Mass Index as an indicator for obesity, it has recently been reported that 59% of American adults exceed a healthy weight. Severe weight loss is a condition which can result from a variety of sources including cancer, AIDS, tissue wasting, anorexia nervosa, chronic infection or gastrointestinal disease. Several genes have been previously reported to be implicated in contributing to obesity in mice (ob, db, tub, A^y and fat).

Summary of the Invention

In one aspect, the invention features a method for determining if an animal is at risk for a body weight disorder. An animal is provided. An aspect of ICAM receptor, e.g., ICAM-1, or β_2 integrin, e.g., Mac-1, metabolism or structure is evaluated in the animal. An abnormality in the aspect of ICAM receptor or β_2 integrin metabolism or structure is diagnostic of being at risk for a body weight disorder.

Another aspect of the invention is a method for detecting the presence of a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal. The level of ICAM receptor or β_2 integrin polypeptide in a

biological sample from a first animal is evaluated. The level obtained in the evaluating step is compared to a level of ICAM receptor or β_2 integrin polypeptide present in a normal second animal or in the first animal at an earlier time. An increase in the level of ICAM receptor or β_2 integrin as compared to a normal level is indicative of a disease affecting body weight associated with elevated levels of ICAM receptor or β_2 integrin polypeptide, and a decreased level of ICAM receptor or β_2 integrin polypeptide as compared to a normal level is indicative of a disease effecting body weight associated with decreased levels of ICAM receptor or β_2 integrin.

In preferred embodiments, the evaluating step comprises contacting the biological sample having ICAM receptor or β_2 integrin polypeptide with an antibody that specifically binds to ICAM receptor or β_2 integrin polypeptide under conditions which allow the formation of reaction complexes comprising the antibody and the ICAM receptor or β_2 integrin polypeptide. The formation of the reaction complexes comprising the antibody and the ICAM receptor or β_2 integrin polypeptide is detected. The amount of the reaction complexes formed is evaluated, the amount corresponding to the level of ICAM receptor or β_2 integrin polypeptide in the biological sample.

Another aspect of the invention is a method for evaluating an agent for use in modulating body weight in an animal. A test cell, cell-free system or animal having a non-wild-type pattern of ICAM receptor or β_2 integrin metabolism is provided. An agent is provided. The agent is administered to the test cell, cell-free system or animal in a therapeutically effective amount. The effect of the agent on an aspect of ICAM receptor or β_2 integrin metabolism or on a parameter related to body weight is evaluated. A change in the aspect of ICAM receptor or β_2 integrin metabolism or the parameter related to body weight is indicative of the usefulness of the agent in modulating body weight in the animal.

In certain embodiments, the method employs two phases for evaluating an agent for use in modulating body weight, an initial in vitro phase and then an in vivo phase. The agent is administered to a test cell or cell-free system in vitro. If a change in the aspect of ICAM receptor or β_2 integrin metabolism occurs, then the agent is further administered to a test animal in a therapeutically effective amount. The in vivo effect of the agent on an aspect of ICAM receptor or β_2 integrin metabolism or a parameter related to body weight is evaluated. A change in the aspect of ICAM receptor or β_2 integrin metabolism or the parameter related to body weight is indicative of the usefulness of the agent in modulating body weight. The test animal can have the same genotype or a different genotype from the test cell or cell-free system.

Another aspect of the invention is a method for evaluating an agent for the ability to modulate body weight in an animal. An agent is provided. ICAM receptor, an extracellular portion of ICAM receptor, β_2 integrin or an extracellular portion of β_2 integrin is provided. The agent is contacted with ICAM receptor, the extracellular portion of ICAM receptor, β_2 integrin or the extracellular portion of β_2 integrin. It is determined if the agent interacts with ICAM receptor, the extracellular portion of ICAM receptor, β_2 integrin or the extracellular portion of β_2 integrin. If interaction is found, then the agent is further administered to a test animal in a therapeutically effective amount. The in vivo effect of the agent on the body weight of the test animal is evaluated.

Another aspect of the invention is a method for evaluating an agent for the ability to modulate body weight in an animal by determining an alteration in the binding of ICAM receptor or β_2 integrin or extracellular portions thereof to a binding molecule. An agent is provided. ICAM receptor or an extracellular portion thereof, or β_2 integrin or an extracellular portion thereof, is provided. A binding molecule or an extracellular portion thereof is provided. The agent, the ICAM receptor or extracellular portion thereof or β_2 integrin or extracellular portion thereof, and the binding molecule or extracellular portion thereof, are combined. The formation of a complex is detected. An alteration in the formation of the complex in the presence of the agent as compared to in the absence of the agent is indicative of the agent altering the binding of the ICAM receptor or extracellular portion thereof or the β_2 integrin or extracellular portion thereof to the binding molecule. If alteration in the formation of the complex occurs, then a test animal is further provided, and the agent is further administered to the test animal in a therapeutically effective amount. The in vivo effect of the agent on the body weight of the test animal is evaluated.

Another aspect of the invention is a method for treating a body weight related disorder in an animal. An animal in need of treatment for a body weight related disorder is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the body weight related disorder occurs.

Another aspect of the invention is a method for treating an animal at risk for a body weight related disorder. An animal at risk for a body weight related disorder is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that

treatment of the animal occurs.

Another aspect of the invention is a method for monitoring a therapeutic treatment of a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal. The levels of ICAM receptor or β_2 integrin polypeptide in a plurality of biological samples obtained at different time points from an animal undergoing a therapeutic treatment for a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide is evaluated.

Another aspect of the invention is a pharmaceutical composition for treating a body weight related disorder in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure in the animal so as to result in treatment of the body weight related disorder in the animal, and a pharmaceutically acceptable carrier.

Another aspect of the invention is a method of making an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal. An ICAM receptor or β_2 integrin polypeptide is provided. The amino acid sequence of the polypeptide is altered. The altered polypeptide is tested for an effect on an aspect of ICAM receptor or β_2 integrin metabolism, a change in the aspect of ICAM receptor or β_2 integrin metabolism being indicative of an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal.

Another aspect of the invention is a method for increasing the fat content of an animal liver, e.g., a goose liver. An animal is provided. Soluble ICAM receptor or soluble β_2 integrin obtained from the animal is administered into the animal so as to increase the fat content of the liver of the animal.

Yet another aspect of the invention is a method for increasing the fat content in milk secreted by an animal. An animal capable of secreting milk is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism is provided. The agent is administered to the animal in an amount so as to increase the fat content in the milk of the animal.

The above and other features, objects and advantages of the present invention will be better understood by a reading of the following specification in conjunction with the drawings.

Brief Description of the Drawings

Fig. 1 depicts growth curves of wild-type and ICAM-1 -/- mice on a normal chow diet (5% fat).

Fig. 2 (a, b, c and d) depicts growth curves of wild-type and ICAM-1 -/- mice on a Western-type diet (21% fat).

Fig. 3 depicts growth curves of wild-type and Mac-1 -/- mice on a Western-type diet (21% fat).

Detailed Description

This invention provides a method for determining if an animal is at risk for a body weight disorder. An animal is provided. An aspect of ICAM receptor or β_2 integrin metabolism or structure is evaluated in the animal. An abnormality in the aspect of ICAM receptor or β_2 integrin metabolism or structure is diagnostic of being at risk for a body weight disorder.

By animal is meant human as well as non-human animals. Non-human animals include, e.g., mammals, e.g., monkeys, chimpanzees, apes, rodents, pigs, rabbits, goats, cows or geese. An animal also includes transgenic non-human animals. The term transgenic animal is meant to include an animal that has gained new genetic information from the introduction of foreign DNA, i.e., partly or entirely heterologous DNA, into the DNA of its cells; or introduction of a lesion, e.g., an in vitro induced mutation, e.g., a deletion or other chromosomal rearrangement into the DNA of its cells; or introduction of homologous DNA into the DNA of its cells in such a way as to alter the genome of the cell into which the DNA is inserted, e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout. The animal may include a transgene in all of its cells including germ line cells, or in only one or some of its cells. Transgenic animals of the invention can serve as a model for studying body weight related disorders. In certain embodiments, the determination for being at risk for body weight related disorders is done in a prenatal animal.

A body weight disorder is meant to include, e.g., obesity or weight loss, e.g., severe weight loss. Obesity is a condition in which there is an excess of body fat. The most common indicator for obesity is Body Mass Index (BMI), which is the individual's weight in kilograms divided by his height in meters squared. BMI is highly correlated with body fat. The 1995 Guidelines from the National Institutes of Health and the American Health Foundation define healthy weight as a BMI below 25. Obesity is often associated with other disease conditions,

e.g., diabetes, high blood pressure and high cholesterol levels.

Weight loss is a condition which can occur as a result of diminished food intake, or in the absence of dietary restriction, e.g., with normal or excessive food intake. Weight loss occurring with normal or excessive food intake can result, e.g., from insulin-dependent diabetes mellitus, thyrotoxicosis or malabsorption of food. Weight loss resulting from diminished food intake can result, e.g., from cancer, AIDS, tissue wasting, anorexia nervosa, chronic infection or gastrointestinal disease. For example, cancer cachexia is a clinical syndrome that develops as a consequence of the nutritional and metabolic abnormalities of the tumor-bearing host, generally with advanced malignancies. This syndrome includes anorexia, body weight loss, severe tissue wasting, asthenia and organ dysfunction. Individuals with malignancies generally have aberrations in lipid metabolism, including an increased rate of lipolysis with accelerated catabolism of body fat stores. During periods of glucose administration, normal individuals suppress lipid mobilization and preferentially oxidize glucose. Individuals with cancer, however, fail to suppress endogenous lipid mobilization, and persistent oxidation of free fatty acids occurs. Relative changes in the proportions of specific lipids contribute to further body compositional and metabolic derangements. Conventional nutrition support generally does not reduce morbidity in cancer patients.

By ICAM receptor (intercellular adhesion molecule) is meant any receptor that is a member of the ICAM family of adhesion receptors. ICAM receptors mediate leukocyte adhesion. ICAM receptors include, e.g., ICAM-1, ICAM-2 and ICAM-3. A preferred ICAM receptor is ICAM-1. ICAM-1 is expressed, e.g., on leukocytes, endothelium, epithelium, hepatocytes, adipocytes, myocytes and fibroblasts. ICAM-1 is a major cell-cell adhesion molecule in inflammatory and immune systems. The primary counterreceptors for ICAM-1 are the β2 integrins, e.g., Mac-1 and LFA-1.

By β_2 integrin is meant any β_2 leukocyte integrin. β_2 integrins include, e.g., Mac-1 and LFA-1. A preferred β_2 integrin is Mac-1 (also referred to as $\alpha_M\beta_2$ or $CD11_b/CD18$). Mac-1 is involved, e.g., in several neutrophil functions including adhesion to the endothelium, phagocytosis and neutrophil apoptosis.

This invention shows that ICAM receptor and β_2 integrin are also involved in the regulation of body weight, e.g., adipose tissue mass. Animals deficient in ICAM receptor or β_2 integrin become obese. ICAM receptor and β_2 integrin play a role in preventing excessive body fat deposition, by regulating lipid metabolism and/or energy expenditure.

By ICAM receptor or β_2 integrin metabolism is meant any aspect of the production, release, expression, function, action, interaction, e.g., cell/cell adhesion, or regulation of ICAM receptor or β_2 integrin. The metabolism of ICAM receptor or β_2 integrin includes modifications, e.g., covalent or non-covalent modifications, of ICAM receptor or β_2 integrin polypeptide. The terms peptides, proteins and polypeptides are used interchangeably herein. The metabolism of ICAM receptor or β_2 integrin includes modifications, e.g., covalent or non-covalent modifications, that ICAM receptor or β_2 integrin induces in other substances. The metabolism of ICAM receptor or β_2 integrin also includes changes in the distribution, concentration, activation or phosphorylation of ICAM receptor or β_2 integrin polypeptide, and changes ICAM receptor or β_2 integrin induces in the distribution, concentration, activation or phosphorylation of other substances.

Any aspect of ICAM receptor or β_2 integrin metabolism can be evaluated. The methods used are standard techniques known to those skilled in the art and can be found in standard references, e.g., Ausubel et al., ed., Current Protocols in Mol. Biology, New York: John Wiley & Sons, 1990; Drewes et al., Mol. and Cell Biol. 16:925-931 (1996). Preferred examples of ICAM receptor or β_2 integrin metabolism that can be evaluated include the binding activity of ICAM receptor or β_2 integrin polypeptide to a binding molecule; the transactivation activity of ICAM receptor or β_2 integrin polypeptide on a target gene; the level of ICAM receptor or β_2 integrin polypeptide; the level of ICAM receptor or β_2 integrin mRNA; or the level of ICAM receptor or β_2 integrin phosphorylation. By binding molecule is meant any molecule to which ICAM receptor or β_2 integrin can bind, e.g., a nucleic acid, e.g., a DNA regulatory region, a protein, a metabolite, a peptide mimetic, a non-peptide mimetic, an antibody, or any other type of ligand. In certain embodiments, the binding molecule itself is ICAM receptor or β_2 integrin, i.e., ICAM receptor, e.g., ICAM-1, can bind to β_2 integrin, e.g., Mac-1. Binding can be shown, e.g., by electrophoretic mobility shift analysis (EMSA). Transactivation of a target gene by ICAM receptor or β_2 integrin can be determined, e.g., in a transient transfection assay in which the promoter of the target gene is linked to a reporter gene, e.g., β-galactosidase or luciferase, and co-transfected with an ICAM receptor or β_2 integrin expression vector. Such evaluations can be done in vitro or in vivo. Levels of ICAM receptor or β_2 integrin protein, mRNA or phosphorylation, can, e.g., be measured in a sample, e.g., a tissue sample, e.g., blood.

In certain embodiments, an aspect of ICAM receptor or β_2 integrin structure is evaluated, e.g., ICAM receptor or β_2 integrin gene structure or ICAM receptor or β_2 integrin protein

structure. For example, primary, secondary or tertiary structures can be evaluated. For example, the DNA sequence of the gene is determined and/or the amino acid sequence of the protein is determined. Standard cloning and sequencing methods can be used as are known to those skilled in the art. In certain embodiments, the binding activity of an antisense nucleic acid with the cellular ICAM receptor or β_2 integrin mRNA and/or genomic DNA is determined using standard methods known to those skilled in the art so as to detect the presence or absence of the target mRNA or DNA sequences to which the antisense nucleic acid would normally specifically bind.

The invention also includes a method for detecting the presence of a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal. The level of ICAM receptor or β_2 integrin polypeptide in a biological sample from a first animal is evaluated. The level obtained in the evaluating step is compared to a level of ICAM receptor or β_2 integrin polypeptide present in a normal second animal or in the first animal at an earlier time. An increase in the level of ICAM receptor or β_2 integrin as compared to a normal level is indicative of a disease affecting body weight associated with elevated levels of ICAM receptor or β_2 integrin polypeptide, and a decreased level of ICAM receptor or β_2 integrin polypeptide as compared to a normal level is indicative of a disease effecting body weight associated with decreased levels of ICAM receptor or β_2 integrin.

In preferred embodiments, the evaluating step comprises contacting the biological sample having ICAM receptor or β_2 integrin polypeptide with an antibody that specifically binds to ICAM receptor or β_2 integrin polypeptide under conditions which allow the formation of reaction complexes comprising the antibody and the ICAM receptor or β_2 integrin polypeptide. The formation of the reaction complexes comprising the antibody and the ICAM receptor or β_2 integrin polypeptide is detected. The amount of the reaction complexes formed is evaluated, the amount corresponding to the level of ICAM receptor or β_2 integrin polypeptide in the biological sample.

Biological sample is meant to include, e.g., blood, leukocytes, endothelium, epithelium, hepatocytes, myocytes, fibroblasts, adipose tissue and lymph. A biological sample is also meant to include samples or portions of the biological sample that have been resuspended in other media or pelleted.

By a normal animal is meant an animal with unimpaired ICAM receptor or β_2 integrin. By a normal level of ICAM receptor or β_2 integrin is meant the level of ICAM receptor or β_2 integrin in a normal animal. For example, the normal level can be ascertained from a separate

normal animal other than the animal being tested, or it can be ascertained from the tested animal from earlier obtained samples when the tested animal was normal.

A disease affecting body weight that is associated with elevated levels of ICAM receptor or β_2 integrin is meant to include, e.g., weight loss related diseases. A disease affecting body weight that is associated with decreased levels of ICAM receptor or β_2 integrin is meant to include, e.g., obesity.

Detection of the reaction complexes formed between the antibodies and ICAM receptor or β_2 integrin can be accomplished by standard methods known to those skilled in the art. For example, the antibodies can be labeled, e.g., with a radioactive label or a non-radioactive label, e.g., fluorescent labels.

The invention also includes a method for evaluating an agent for use in modulating body weight in an animal. A test cell, cell-free system or animal having a non-wild-type pattern of ICAM receptor or β_2 integrin metabolism is provided. An agent is provided. The agent is administered to the test cell, cell-free system or animal in a therapeutically effective amount. The effect of the agent on an aspect of ICAM receptor or β_2 integrin metabolism or on a parameter related to body weight is evaluated. A change in the aspect of ICAM receptor or β_2 integrin metabolism or the parameter related to body weight is indicative of the usefulness of the agent in modulating body weight in the animal.

In certain embodiments, the method employs two phases for evaluating an agent for use in modulating body weight, an initial in vitro phase and then an in vivo phase. The agent is administered to a test cell or cell-free system in vitro. If a change in the aspect of ICAM receptor or β_2 integrin metabolism occurs, then the agent is further administered to a test animal in a therapeutically effective amount. The in vivo effect of the agent on an aspect of ICAM receptor or β_2 integrin metabolism or a parameter related to body weight is evaluated. A change in the aspect of ICAM receptor or β_2 integrin metabolism or the parameter related to body weight is indicative of the usefulness of the agent in modulating body weight. The test animal can have the same genotype or a different genotype from the test cell or cell-free system.

Modulating body weight is meant to include increasing body weight or decreasing body weight.

By cell is meant a cell or a group of cells, or a cell that is part of an animal. The cell can be a human or non-human cell. Cell is also meant to include a transgenic cell. The cell can be obtained, e.g., from a culture or from an animal. The animal can be a natural animal or non-

human transgenic animal. In certain embodiments, the transgenic cell or non-human transgenic animal has an ICAM receptor or β_2 integrin transgene, or fragment or analog thereof. In certain embodiments, the transgenic cell or non-human transgenic animal has a deletion, e.g., a knockout, or an addition of a gene coding for ICAM receptor or β_2 integrin.

A non-wild-type pattern of ICAM receptor or β_2 integrin metabolism can result, e.g., from underexpression, overexpression, no expression, or a temporal, site or distribution change in expression. Such a non-wild-type pattern can result, e.g., from one or more mutations in the ICAM receptor or β_2 integrin gene, in a binding molecule gene, or in any other gene which directly or indirectly affects ICAM receptor or β_2 integrin metabolism. A mutation is meant to include, e.g., an alteration, e.g., in gross or fine structure, in a nucleic acid. Examples include single base pair alterations, e.g., missense or nonsense mutations, frameshifts, deletions, insertions and translocations. Mutations can be dominant or recessive. Mutations can be homozygous or heterozygous.

Any aspect of ICAM receptor or β_2 integrin metabolism can be evaluated. In certain embodiments, the aspect of metabolism is the binding of ICAM receptor to β_2 integrin. Any parameter related to body weight can be evaluated. In certain embodiments, the parameter is lipid metabolism, e.g., regulation of fatty acid oxidation.

An agent is meant to include, e.g., any substance, e.g., a drug for weight gain or a drug for weight loss. The agent of this invention preferably can change an aspect of ICAM receptor or β_2 integrin metabolism. Such change can be the result of any of a variety of events, including, e.g., preventing or reducing interaction between ICAM receptor or β_2 integrin and a binding molecule; inactivating ICAM receptor or β_2 integrin and/or the binding molecule, e.g., by cleavage or other modification; altering the affinity of ICAM receptor or β_2 integrin and the binding molecule for each other; diluting out ICAM receptor or β_2 integrin and/or the binding molecule; preventing expression of ICAM receptor or β_2 integrin and/or the binding molecule; reducing synthesis of ICAM receptor or β_2 integrin and/or the binding molecule; synthesizing an abnormal ICAM receptor or β_2 integrin and/or binding molecule; synthesizing an alternatively spliced ICAM receptor or β_2 integrin and/or binding molecule; preventing or reducing proper conformational folding of ICAM receptor or β_2 integrin and/or the binding molecule; modulating the binding properties of ICAM receptor or β_2 integrin and/or the binding molecule; interfering with signals that are required to activate or deactivate ICAM receptor or β_2 integrin and/or the binding molecule; activating or deactivating ICAM receptor or β_2 integrin and/or the binding

molecule at the wrong time; or interfering with other receptors, ligands or other molecules which are required for the normal synthesis or functioning of ICAM-1 or Mac-1 and/or the binding molecule. The binding molecule can be on the same or different cell as ICAM or β_2 integrin.

Examples of agents include ICAM receptor or β_2 integrin polypeptide or a biologically active fragment or analog thereof; a nucleic acid encoding ICAM receptor or β_2 integrin polypeptide or a biologically active fragment thereof; a nucleic acid encoding an ICAM receptor or β_2 integrin regulatory sequence or a biologically active fragment thereof; a binding molecule for ICAM receptor or β_2 integrin polypeptide; a binding molecule for ICAM receptor or β_2 integrin nucleic acid, the ICAM receptor or β_2 integrin nucleic acid being, e.g., a nucleic acid comprising a regulatory region for ICAM receptor or β_2 integrin or a nucleic acid comprising a structural region for ICAM receptor or β_2 integrin or a biologically active fragment of ICAM receptor or β_2 integrin; an antisense nucleic acid; a mimetic of ICAM receptor or β_2 integrin or a binding molecule; an antibody for ICAM receptor or β_2 integrin or a binding molecule; a metabolite; or an inhibitory peptide, carbohydrate or glycoprotein. In certain embodiments, the agent is an antagonist, agonist or super agonist.

ICAM receptor or β_2 integrin allows a search for natural or artificial ligands to regulate fat metabolism in the treatment of body weight disorders. In certain embodiments, the agent is a natural ligand for ICAM receptor or β_2 integrin. In certain embodiments, the agent is an artificial ligand for ICAM receptor or β_2 integrin.

By analog is meant a compound that differs from naturally occurring ICAM receptor or β_2 integrin in amino acid sequence or in ways that do not involve sequence, or both. Analogs of the invention generally exhibit at least about 90% homology, preferably at least about 95% homology, and most preferably at least about 99% homology, with a segment of 20 amino acid residues, preferably with more than 40 amino acid residues, or more preferably yet with substantially the entire sequence of a naturally occurring ICAM receptor or β_2 integrin sequence. Non-sequence modifications include, e.g., in vivo or in vitro chemical derivatizations of ICAM receptor or β_2 integrin. Non-sequence modifications include, e.g., changes in phosphorylation, acetylation, methylation, carboxylation, or glycosylation. Methods for making such modifications are known to those skilled in the art. For example, phosphorylation can be modified by exposing ICAM receptor or β_2 integrin to phosphorylation-altering enzymes, e.g., kinases or phosphatases.

Preferred analogs include ICAM receptor or β_2 integrin or biologically active fragments

thereof, whose sequences differ from the wild-type sequence by one or more conservative amino acid substitutions or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish ICAM receptor or β_2 integrin biological activity. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g., substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Other conservative substitutions are shown in Table 1.

TABLE 1

Conservative Amino Acid Substitutions

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, β-Ala Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Leu, D-Leu, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Amino acid sequence variants of a protein can be prepared by any of a variety of methods known to those skilled in the art. For example, random mutagenesis of DNA which encodes a protein or a particular domain or region of a protein can be used, e.g., PCR mutagenesis (using, e.g., reduced *Taq* polymerase fidelity to introduce random mutations into a cloned fragment of DNA; Leung et al., Technique 1:11-15 (1989)), or saturation mutagenesis (by, e.g., chemical treatment or irradiation of single-stranded DNA in vitro, and synthesis of a complementary DNA strand; Myers et al., Science 229:242-247 (1985)). Random mutagenesis can also be accomplished by, e.g., degenerate oligonucleotide generation (using, e.g., an automatic DNA synthesizer to chemically synthesize degenerate sequences; Narang, Tetrahedron 39:3 (1983); Itakura et al., Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. A.G. Walton, Amsterdam: Elsevier, pp. 273-289 (1981)). Non-random or directed mutagenesis can be used to provide specific sequences or mutations in specific regions. These techniques can be used to create variants which include, e.g., deletions, insertions, or substitutions, of residues of the known amino acid sequence of a protein. The sites for mutation can be modified individually or in series, e.g., by (i) substituting first with conserved amino acids and then with more radical choices depending upon results achieved, (ii) deleting the target residue, (iii) inserting residues of the same or a different class adjacent to the located site, or (iv) combinations of the above.

Methods for identifying desirable mutations include, e.g., alanine scanning mutagenesis (Cunningham and Wells, Science 244:1081-1085 (1989)), oligonucleotide-mediated mutagenesis (Adelman et al., DNA 2:183-193 (1983)); cassette mutagenesis (Wells et al., Gene 34:315-323 (1985)), combinatorial mutagenesis, and phage display libraries (Ladner et al., WO88/06630).

Other analogs within the invention include, e.g., those with modifications which increase peptide stability. Such analogs may contain, e.g., one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are, e.g.: analogs that include residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids; and cyclic analogs. Analogs can be made by methods known to those skilled in the art.

By fragment is meant some portion of the naturally occurring ICAM receptor or β_2 integrin polypeptide. Preferably, the fragment is at least about 3 amino acid residues, more preferably at least about 10 to at least about 20 amino acid residues. In certain embodiments, entire domains containing at least about 50 amino acids are used. Fragments include, e.g., truncated secreted forms, proteolytic fragments, splicing fragments, other fragments, and

chimeric constructs between at least a portion of the relevant gene, e.g., ICAM receptor or β_2 integrin, and another molecule. Fragments of ICAM receptor or β_2 integrin can be generated by methods known to those skilled in the art. The ability of a candidate fragment to exhibit a biological activity of ICAM receptor or β_2 integrin can be assessed by methods known to those skilled in the art. Also included are ICAM receptor or β_2 integrin fragments containing residues that are not required for biological activity of the fragment or that result from alternative mRNA splicing or alternative protein processing events.

Fragments of a protein can be produced in several ways, e.g., recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments. Digestion with "end-nibbling" endonucleases can thus generate DNAs which encode an array of fragments. DNAs which encode fragments of a protein can also be generated, e.g., by random shearing, restriction digestion or a combination of the above-discussed methods. For example, fragments of ICAM receptor or β_2 integrin can be made by expressing ICAM receptor or β_2 integrin DNA which has been manipulated in vitro to encode the desired fragment, e.g., by restriction digestion of the DNA sequence for the gene.

Fragments can also be chemically synthesized using techniques known in the art, e.g., conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, peptides of the present invention can be arbitrarily divided into fragments of desired length with no overlap of the fragments, or divided into overlapping fragments of a desired length.

ICAM receptor or β_2 integrin or a biologically active fragment or analog thereof, or a binding molecule or a biologically active fragment or analog thereof, can, e.g., compete with its cognate molecule for the binding site on the complementary molecule, and thereby reduce or eliminate binding between ICAM receptor or β_2 integrin and the binding molecule. ICAM receptor or β_2 integrin or a binding molecule can be obtained, e.g., from purification or secretion of naturally occurring ICAM receptor or β_2 integrin or a binding molecule, from recombinant ICAM receptor or β_2 integrin or a binding molecule, or from synthesized ICAM receptor or β_2 integrin or a binding molecule.

Therefore, methods for generating analogs and fragments and testing them for activity are known to those skilled in the art.

An agent can also be a nucleic acid used as an antisense molecule. Antisense therapy is meant to include, e.g., administration or in situ generation of oligonucleotides or their derivatives which specifically hybridize, e.g., bind, under cellular conditions, with the cellular mRNA and/or genomic DNA encoding an ICAM receptor or β_2 integrin polypeptide, or mutant thereof, so as to inhibit expression of the encoded protein, e.g., by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix.

In certain embodiments, the antisense construct binds to a naturally-occurring sequence of an ICAM receptor or β_2 integrin gene which, e.g., is involved in expression of the gene. These sequences include, e.g., start codons and stop codons.

In other embodiments, the antisense construct binds to a nucleotide sequence which is not present in the wild-type gene. For example, the antisense construct can bind to a region of an ICAM receptor or β_2 integrin gene which contains an insertion of an exogenous, non-wild-type sequence. Alternatively, the antisense construct can bind to a region of an ICAM receptor or β_2 integrin gene which has undergone a deletion, thereby bringing two regions of the gene together which are not normally positioned together and which, together, create a non-wild-type sequence. When administered in vivo to a subject, antisense constructs which bind to non-wild-type sequences provide the advantage of inhibiting the expression of a mutant ICAM receptor or β_2 integrin gene, without inhibiting expression of any wild-type ICAM receptor or β_2 integrin gene.

An antisense construct of the present invention can be delivered, e.g., as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular mRNA which encodes an ICAM receptor or β_2 integrin polypeptide. An alternative is that the antisense construct is an oligonucleotide probe which is generated ex vivo and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of an ICAM receptor or β_2 integrin gene. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, and are therefore stable in vivo. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphorothioate and methylphosphonate analogs of DNA. (See also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed. (See, e.g., van der Krol and Molo, Biotechniques 6:958-

976, (1988); Stein and Cohen, Cancer Res. 48:2659-2668 (1988)). Examples of antisense oligonucleotides include phosphorothioate antisense oligonucleotides which target the AUG translation initiation codon or sequences in the 3'- untranslated region of ICAM-1 message (Chiang et al., J. Biol. Chem. 266:18162-18171 (1991); antisense oligonucleotides to ICAM-1 3'- untranslated region (Kumaska et al., J. Clin. Invest. 97:2362-2369 (1996)).

By mimetic is meant a molecule which resembles in shape and/or charge distribution ICAM receptor or β_2 integrin or a binding molecule. The mimetic can be a peptide or a non-peptide. Mimetics can act as therapeutic agents because they can, e.g., competitively inhibit binding of ICAM receptor or β_2 integrin to a binding molecule. By employing, e.g., scanning mutagenesis, e.g., alanine scanning mutagenesis, linker scanning mutagenesis or saturation mutagenesis, to map the amino acid residues of a particular ICAM receptor or β_2 integrin polypeptide involved in binding a binding molecule, peptide mimetics, e.g., diazopine or isoquinoline derivatives, can be generated which mimic those residues in binding to a binding molecule, and which therefore can inhibit binding of the ICAM receptor or β_2 integrin to a binding molecule and thereby interfere with the function of ICAM receptor or β_2 integrin. For example, non-hydrolyzable peptide analogs of such residues can be generated using benzodiazepine (see, e.g., Freidinger et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); azepine (see, e.g., Huffman et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); substituted gamma lactam rings (see, e.g., Garvey et al., in Peptides: Chemistry and Biology, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands (1988)); keto-methylene pseudopeptides (see, e.g., Ewenson et al., J. Med. Chem. 29:295 (1986); Ewenson et al., in Peptides: Structure and Function (Proceedings of the 9th American Peptide Symposium) Pierce Chemical Co. Rockland, IL (1985)); β-turn dipeptide cores (see, e.g., Nagai et al., Tetrahedron Lett. 26:647 (1985); Sato et al., J. Chem. Soc. Perkin Trans. 1:1231 (1986)); or β-aminoalcohols (see, e.g., Gordon et al., Biochem. Biophys. Res. Commun. 126:419-426 (1985); Dann et al., Biochem. Biophys. Res. Commun. 134:71-77 (1986)).

Protein or peptide ligand inhibitors of Mac-1 have been described. For example, NIF Neutrophil Adhesion Inhibitor is a naturally occurring 41kDa glycoprotein derived from hookworm. (Horton, M.A. (ed.), Adhesion Receptors as Therapeutic Targets, Boca Raton:CRC Press (1996)). In certain embodiments, peptides and mimetics of the active epitope of this protein can be administered orally. Peptides derived from fibrinogen (specifically γ chain) have

been shown to bind to the I-domain of Mac-1 and inhibit its function (Wright et al., Proc. Natl. Acad. Sci. USA 85:7734-7738 (1988); Altieri et al., J. Biol. Chem. 268:1847-1853 (1993)). Non-peptide inhibitors of Mac-1 have been described, including leumedins (N-[9H-(2,7-dimethylfluorenyl-9-methoxy)carbonyl]-leucine, NPC 15669) (Bator et al., Immunopharmacology 23:139 (1992); Burch et al., J. Immunology 150:3397-3403 (1993)).

Antibodies are meant to include antibodies against any moiety that directly or indirectly affects ICAM receptor or β_2 integrin metabolism. The antibodies can be directed against, e.g., ICAM receptor or β_2 integrin or a binding molecule, or a subunit or fragment thereof. For example, antibodies include anti-ICAM receptor or anti-β_2 integrin antibodies; anti-binding molecule antibodies; and Fab_2' fragments of the inhibitory antibody generated through, e.g., enzymatic cleavage. Both polyclonal and monoclonal antibodies can be used in this invention. Preferably, monoclonal antibodies are used. Most preferably, the antibodies have a constant region derived from a human antibody and a variable region derived from an inhibitory mouse monoclonal antibody. Examples of antibodies to Mac-1 include monoclonal antibodies to αM (CD11b, part of Mac-1) (Simpson et al., J. Clin. Invest. 81:624-629 (1988); Hill et al., Surgery 112:166-172 (1992); and anti-β_2 monoclonal antibodies (Vedder et al., Surgery 106:509-516 (1989); Vedder et al., Proc. Natl. Acad. Sci. USA 81:7538-7542 (1990); Mileski et al., Surgery 108:206-212 (1990); Hill et al., J. Appl. Physiol. 74:659-664 (1993)). Examples of antibodies to LFA-1 include antibodies to the related integrin subunit αL (CD11a, part of LFA-1 (Fisher et al., Blood 77:249-256 (1991)). Examples of monoclonal antibodies to ICAM-1 are described in Bowes et al., Exp. Neurol. 119:215-219 (1993); Seecamp et al., Am. J. Pathol. 143:464-472 (1993). Human monoclonal antibodies for ICAM-1 and Mac-1 are commercially available, e.g., from R&D Systems, Minneapolis, MN.

Agents also include inhibitors of a molecule that are required for synthesis, post-translational modification, or functioning of ICAM receptor or β_2 integrin and/or a binding molecule, or activators of a molecule that inhibits the synthesis or functioning of ICAM receptor or β_2 integrin and/or the binding molecule. Agents include, e.g., cytokines, growth factors, hormones, signaling components, kinases, phosphatases, homeobox proteins, transcription factors, translation factors and post-translation factors or enzymes. Agents are also meant to include ionizing radiation, non-ionizing radiation, ultrasound and toxic agents which can, e.g., at least partially inactivate or destroy ICAM-1 or Mac-1 and/or the binding molecule.

An agent is also meant to include agents which are not entirely ICAM receptor or β_2

integrin specific. For example, an agent may alter other lipid metabolism related genes or proteins. Such overlapping specificity may provide additional therapeutic advantage.

The invention also includes the agent so identified as being useful in modulating body weight.

The invention also includes a method for evaluating an agent for the ability to modulate body weight in an animal. An agent is provided. ICAM receptor, an extracellular portion of ICAM receptor, β_2 integrin or an extracellular portion of β_2 integrin is provided. The agent is contacted with ICAM receptor, the extracellular portion of ICAM receptor, β_2 integrin or the extracellular portion of β_2 integrin. It is determined if the agent interacts with ICAM receptor, the extracellular portion of ICAM receptor, β_2 integrin or the extracellular portion of β_2 integrin. If interaction is found, then the agent is further administered to a test animal in a therapeutically effective amount. The in vivo effect of the agent on the body weight of the test animal is evaluated.

In certain embodiments, determining whether interaction has occurred comprises determining whether binding has occurred between the agent and the compound. In certain embodiments, the test agent is administered more than one time to the test animal.

The invention also includes a method for evaluating an agent for the ability to modulate body weight in an animal by determining an alteration in the binding of ICAM receptor or β_2 integrin or extracellular portions thereof to a binding molecule. An agent is provided. ICAM receptor or an extracellular portion thereof or β_2 integrin or an extracellular portion thereof is provided. A binding molecule or an extracellular portion thereof is provided. The agent, the ICAM receptor or extracellular portion thereof or β_2 integrin or an extracellular portion thereof, and the binding molecule or extracellular portion thereof, are combined. The formation of a complex comprising the ICAM receptor or extracellular portion thereof and the binding molecule or extracellular portion thereof, or the β_2 integrin or extracellular portion thereof and the binding molecule or extracellular portion thereof, is detected. An alteration in the formation of the complex in the presence of the agent as compared to in the absence of the agent is indicative of the agent altering the binding of the ICAM receptor or extracellular portion thereof or the β_2 integrin or extracellular portion thereof to the binding molecule. If alteration in the formation of the complex occurs, then a test animal is further provided, and the agent is further administered to the test animal in a therapeutically effective amount. The in vivo effect of the agent on the body weight of the test animal is evaluated.

Altering the binding includes, e.g., inhibiting or promoting the binding. In certain embodiments, the binding molecule is β_2 integrin. In certain embodiments, the binding molecule is ICAM receptor. The efficacy of the agent can be assessed, e.g., by generating dose response curves from data obtained using various concentrations of the agent. Methods for determining formation of a complex are standard and are known to those skilled in the art.

The invention also includes the agent so identified as being able to alter the binding of ICAM receptor or β_2 integrin polypeptide to a binding molecule.

The invention also includes a method for treating a body weight related disorder in an animal. An animal in need of treatment for a body weight related disorder is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the body weight related disorder occurs.

The body weight related disorder can be, e.g., obesity or weight loss. In certain embodiments, the obesity is associated with diabetes, high blood pressure or high cholesterol levels. In certain embodiments, the weight loss results from cancer, AIDS, tissue wasting, anorexia nervosa, chronic infection, gastrointestinal disease, insulin-dependent diabetes mellitus, thyrotoxicosis or malabsorption of food.

Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the body weight related disorder.

The agent can be, e.g., ICAM receptor or β_2 integrin polypeptide or a biologically active fragment or analog thereof; a nucleic acid encoding ICAM receptor or β_2 integrin polypeptide or a biologically active fragment thereof; a nucleic acid encoding an ICAM receptor or β_2 integrin regulatory sequence or a biologically active fragment thereof; a binding molecule for ICAM receptor or β_2 integrin polypeptide; a binding molecule for ICAM receptor or β_2 integrin nucleic acid, the ICAM receptor or β_2 integrin nucleic acid being, e.g., a nucleic acid comprising a regulatory region for ICAM receptor or β_2 integrin or a nucleic acid comprising a structural region for ICAM receptor or β_2 integrin or a biologically active fragment of ICAM receptor or β_2 integrin; an antisense nucleic acid; a mimetic of ICAM receptor or β_2 integrin or a binding molecule; an antibody for ICAM receptor or β_2 integrin or a binding molecule; a metabolite; or an inhibitory carbohydrate or glycoprotein. In certain embodiments, the agent is an antagonist, agonist or super agonist.

For body weight disorders involving underexpression or no expression of ICAM receptor

or β_2 integrin, that result in obesity, it is preferred to add more ICAM receptor or β_2 integrin, either directly or indirectly. For example, addition of ICAM receptor or β_2 integrin can result from addition of ICAM receptor or β_2 integrin polypeptide, or addition of the ICAM receptor or β_2 integrin gene, or from up-regulation of the ICAM receptor or β_2 integrin gene, or from addition of an agonist of ICAM receptor or β_2 integrin. In certain embodiments, the ICAM receptor or β_2 integrin genes can be modified so as to give higher expression. Such modification can be done in vitro or in vivo by standard techniques known to those skilled in the art.

For body weight disorders involving overexpression of ICAM receptor or β_2 integrin, that result in a weight loss disorder, it is preferred to inhibit the ICAM receptor or β_2 integrin polypeptide, either directly or indirectly. For example, addition of an inhibitor of ICAM receptor or β_2 integrin, e.g., an antagonist, e.g., an antibody for ICAM receptor or β_2 integrin, an ICAM receptor or β_2 integrin polypeptide fragment or analog, a mimetic of ICAM receptor or β_2 integrin or an antisense molecule for ICAM receptor or β_2 integrin, or an inhibitor of a molecule, e.g., a cytokine, that induces the expression, e.g., the overexpression of ICAM receptor or β_2 integrin. For example, certain fragments or analogs of ICAM receptor or β_2 integrin can compete with the cognate molecule for the binding site on a complementary molecule, and thereby reduce or eliminate binding between ICAM receptor or β_2 integrin and their binding molecules. Administration of the agent can be accomplished by any method which allows the agent to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the agent is obtained. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

Administration of the agent can be alone or in combination with other therapeutic agents. In certain embodiments, the agent can be combined with a suitable carrier, incorporated into a liposome, or incorporated into a polymer release system.

In certain embodiments of the invention, the administration can be designed so as to

result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the animal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time period. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches or sub-cutaneous implants.

Examples of systems in which release occurs in bursts include, e.g., systems in which the agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimuli, e.g., temperature, pH, light or a degrading enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the agent is gradual and continuous include, e.g., erosional systems in which the agent is contained in a form within a matrix, and diffusional systems in which the agent permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The agent can be suspended in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

The agent can be administered prior to or subsequent to the appearance of body weight related disorder symptoms. In certain embodiments, the agent is administered to patients with familial histories of body weight related disorders, or who have phenotypes that may indicate a predisposition to a body weight related disorder, or who have been diagnosed as having a genotype which predisposes the patient to a body weight related disorder, or who have a disease which is associated with a body weight related disorder.

The agent is administered to the animal in a therapeutically effective amount. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing the body weight related disorder. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the species of animal, the animal's size, the animal's age, the agent used, the type of delivery system used, the time of administration relative to the onset of body weight related disorder symptoms,

and whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Preferably, the concentration of the agent is at a dose of about 0.1 to about 1000 mg/kg body weight, more preferably at about 0.1 to about 500 mg/kg, more preferably yet at about 0.1 to about 100 mg/kg, and most preferably at about 0.1 to about 5 mg/kg. The specific concentration partially depends upon the particular agent used, as some are more effective than others. The dosage concentration of the agent that is actually administered is dependent at least in part upon the final concentration that is desired at the site of action, the method of administration, the efficacy of the particular agent, the longevity of the particular agent, and the timing of administration relative to the onset of the body weight related disorder symptoms. Preferably, the dosage form is such that it does not substantially deleteriously affect the animal. The dosage can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In certain embodiments, various gene constructs can be used as part of a gene therapy protocol to deliver nucleic acids encoding, e.g., either an agonistic or antagonistic form of an ICAM receptor or β_2 integrin polypeptide or their binding molecules. Expression vectors can be used for in vivo transfection and expression of an ICAM receptor or β_2 integrin polypeptide in particular cell types so as to reconstitute the function of, or alternatively, abrogate the function of ICAM receptor or β_2 integrin polypeptide in a cell in which non-wild-type ICAM receptor or β_2 integrin is expressed. Expression constructs of the ICAM receptor or β_2 integrin polypeptide, and mutants thereof, may be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the ICAM receptor or β_2 integrin gene to cells in vivo. Approaches include, e.g., insertion of the subject gene in viral vectors including, e.g., recombinant retroviruses, adenovirus, adeno-associated virus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out in vivo or in vitro. The above-described methods are known to those skilled in the art and can be performed without undue experimentation. Since transduction of appropriate target cells represents the critical first step in gene therapy, choice of the particular

gene delivery system will depend on such factors as the phenotype of the intended target and the route of administration, e.g., locally or systemically. Administration can be directed to one or more cell types, and to one or more cells within a cell type, so as to be therapeutically effective, by methods that are known to those skilled in the art. In preferred embodiments, the agent is administered to the liver or adipose tissue of the animal. In another preferred embodiment, the agent is administered to a stem cell of the animal. For example, a genetically engineered ICAM receptor or β_2 integrin is administered to the liver or to hematopoietic stem cells by a viral vector. In certain embodiments, administration is done in a prenatal animal or embryonic cell. It will be recognized that the particular gene construct provided for in in vivo transduction of ICAM receptor or β_2 integrin expression are also useful for in vitro transduction of cells, such as for use in the diagnostic assays described above.

The invention also includes a method for treating an animal at risk for a body weight related disorder. An animal at risk for a body weight related disorder is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism is provided. The agent is administered to the animal in a therapeutically effective amount such that treatment of the animal occurs. Being at risk for a body weight related disorder can result from, e.g., a familial history of a body weight related disorder, phenotypic symptoms which predispose to a body weight related disorder, a genotype which predisposes to a body weight related disorder, or having a disease which is associated with a body weight related disorder.

The invention also includes a method for monitoring a therapeutic treatment of a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal. The levels of ICAM receptor or β_2 integrin polypeptide in a plurality of biological samples obtained at different time points from an animal undergoing a therapeutic treatment for a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide is evaluated.

The invention also includes a pharmaceutical composition for treating a body weight related disorder in an animal comprising a therapeutically effective amount of an agent, the agent being capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure in the animal so as to result in treatment of the body weight related disorder in the animal, and a pharmaceutically acceptable carrier.

In certain embodiments, the agent is an antagonist of ICAM receptor or β_2 integrin, e.g., an antibody for ICAM receptor or β_2 integrin, a fragment or analog of ICAM receptor or β_2

integrin, a small molecule antagonist of ICAM receptor or β_2 integrin, a mimetic of ICAM receptor or β_2 integrin, an antisense molecule for ICAM receptor or β_2 integrin, or a binding molecule for ICAM receptor or β_2 integrin.

In certain embodiments, the agent is an agonist or super agonist of ICAM receptor or β_2 integrin.

The invention also includes a method of making an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal. An ICAM receptor or β_2 integrin polypeptide is provided. The amino acid sequence of the polypeptide is altered. The altered polypeptide is tested for an effect on an aspect of ICAM receptor or β_2 integrin metabolism, a change in the aspect of ICAM receptor or β_2 integrin metabolism being indicative of an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal. The polypeptides can be generated and tested for an effect on an aspect of ICAM receptor or β_2 integrin metabolism by methods known to those skilled in the art, e.g., as described herein. In certain embodiments, the altered polypeptide is further tested in an animal to determine if the altered polypeptide modulates the body weight of the animal.

The invention also includes a method for increasing the fat content of an animal liver. An animal is provided. Soluble ICAM receptor or soluble β_2 integrin obtained from the animal is administered into the animal so as to increase the fat content of the liver in the animal. In a preferred embodiment, the animal is a goose. In certain embodiments, the animal is engineered to lack ICAM-1. Such a method can be used in the food industry to produce, e.g., fatty goose livers for paté.

The invention also includes a method for increasing the fat content in milk secreted by an animal. An animal capable of secreting milk is provided. An agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism is provided. The agent is administered to the animal in an amount so as to increase the fat content in the milk of the animal. Preferably, the animal is a livestock milk-producing animal. In certain embodiments, the animal is a non-human transgenic lactating animal. A preferred animal is a goat. Since certain membrane proteins are found on fat mass envelopes in secreted milk, by increasing fat production in milk, the amount of the membrane protein on the secreted fat is increased. Such a method is therefore useful, e.g., for increasing the yield per animal of a desired membrane protein.

The following non-limiting examples further illustrate the present invention.

EXAMPLES

Example 1: Growth of wild-type and ICAM-1 -/- mice on a normal chow diet (5% fat)

This example illustrates that after 16 weeks of age, ICAM-1 -/- mice gained more weight than wild-type mice on a normal chow diet (5% fat), and thus became spontaneously obese.

The animals used were ICAM-1 -/- mice on a C57BL/6 background. The mice were maintained on 12-h dark and 12-h light cycles. Water and food were available ad libitum. The mouse food was mouse chow (Prolab 3000; PMI Feeds, Inc. St. Louis, MO) which contained 5.0% (wt/wt) fat, 5% (wt/wt) fiber and 22% (wt/wt) protein.

Growth curves of wild-type and ICAM-1 -/- mice on a normal chow diet were determined. See Fig. 1. Each group contained 13 to 14 male C57BL/6 mice. Data were combined from two independent experiments showing similar results. A limited number of female mice were examined and displayed a similar growth difference between ICAM-1 -/- and wild-type (23.57 ± 0.64 g in ICAM-1 -/- vs. 23.95 ± 0.44 g in wild-type at 12 weeks of age, $p=0.64$; 39.87 ± 2.54 g vs. 33.02 ± 1.2 g at 24 weeks of age, $p<0.05$; 54.10 ± 6.20 g vs. 36.70 ± 1.20 g at 45 weeks of age, $p<0.03$. *$p<0.004$; **$p=0.05$.

On normal chow diet, ICAM-1 -/- mice maintained a body weight comparable to wild-type animals until 16 weeks of age. Thereafter, it was observed that ICAM-1 -/- mice gained more weight than control mice. Compared with other genetically obese mice, the growth rate of ICAM-1 -/- mice was similar to that of Tubby mice, except that the weight gain of Tubby mice occurs earlier (at 9-12 week-old). At 24 weeks of age, the average body weight of ICAM-1 -/- and Tubby mice were similar (46 g in Tubby males vs. 44 g in ICAM-1 -/- males; compared to 36 g in wild-type controls). However, unlike Tubby mice which gradually ate more food than controls, it was found that consumption of chow food by ICAM-1 -/- and control mice were comparable (20-25 g/two weeks, at 16 to 24 weeks of age).

Most of the excess body weight in ICAM-1 -/- mice was due to the increased weight of fat-pad (see Table 2). Data are presented as mean ± standard error of the mean. Statistical significance was assessed by Student's test.

TABLE 2

Characteristics of ICAM-1 -/- mice on chow diet

	Body weight (g)	Body mass index (g/cm^2)	White fat-pad weight (% body weight)	Brown fat-pad weight (g)
Wild-type	36.06 ± 1.19	0.40 ± 0.01	9.89 ± 0.65	0.27 ± 0.04
ICAM-1 -/-	43.74 ± 1.60	0.49 ± 0.01	20.1 ± 0.69	0.43 ± 0.05
% of wild-type	121% p<0.001	123% p<0.0001	203% p<0.0001	159% p<0.02

Each group contained 12 to 14 male C57BL/6 mice at 24 weeks of age. Mice were given a standard chow diet. Body-mass-index (BMI) was calculated as body weight (g) divided by the square of body length (anal-nasal length, cm). White fat-pad included the subcutaneous, inguinal, omental and retro-peritoneal fat-pad. Brown fat-pad was taken from the interscapular site.

The 5.2 g excess in white fat (8.81 ± 0.47 g in ICAM-1 -/- mice vs. 3.66 ± 0.36 g in wild-type mice, p<0.0001) was composed predominantly of subcutaneous fat (54%). The weight of interscapular brown fat-pad was also significantly increased in ICAM-1 -/- mice. Brown adipose tissue is an important site of facultative energy expenditure and functions to prevent obesity. Therefore, the increase of brown adipose tissue in obese ICAM-1 -/- mice may be secondary to increased white adipose tissue weight as a compensatory factor protecting against further white fat deposition, or it may reflect impairment in function of brown fat tissue resulting in cellular enlargement with accumulation of more lipid vacuoles. The weights of kidney, spleen and heart in the 24 week-old ICAM-1 -/- mice were similar to those of wild-type mice. However, ICAM-1 -/- mice tended to have a slightly heavier liver (1.82 ± 0.13 g vs. 1.53 ± 0.09 g, p=0.06). Haematoxylin-stained liver sections conterstained with oil-red-O revealed abnormal lipid deposits in the hepatocytes of ICAM-1 -/- mice, indicating that these mice develop fatty livers.

Example 2: Growth of wild-type and ICAM-1 -/- mice on a Western-type diet (21% fat)

This example illustrates that ICAM-1 -/- mice rapidly gained more weight than wild-type mice when fed a Western-type diet containing 21% fat, and thus are sensitive to diet-induced obesity.

Seven week old ICAM-1 -/- and wild-type control mice were given a Western-type diet containing 21% fat. The Western-type diet (Harlan Teklad Adjusted Calories Western-Type Diet No. 88137, Madison, WI) contained 21% (wt/wt) fat (42% of calories), 49.2% (wt/wt) carbohydrate and 19.8% (wt/wt) protein. See Fig. 2. Growth curves (a, b) and weight gains per two weeks (c, d) were determined for wild-type and ICAM-1 -/- mice on Western-type diet. a and c are male mice, b and d are female mice. 9 and 11 C57BL/6 mice in each group were fed Western-type diet starting at 7 weeks of age. Body weight gain (grams per mouse) was measured at two week intervals. "0-2" refers to feeding time from the start of the diet to 2 weeks of feeding, and so forth. The experiment for female groups was repeated with similar results. a, from 2 to 18 weeks $p<0.02$; b, from 4 to 18 weeks $p<0.01$; c and d, $*p<0.05$.

Both male and female mutant mice rapidly gained more body weight than controls (Fig. 2, a and b). This indicates that ICAM-1 -/- mice are susceptible to diet-induced obesity.

There was a difference between male and female ICAM-1 -/- mice in their capacity to regulate body weight gain induced by the Western-type diet. After 6 weeks on a Western-type diet, ICAM-1 -/- male mice no longer gained more weight than controls (Fig. 2c). Therefore, the maximal difference in body weight between male ICAM-1 -/- and wild-type mice was reached by 6 weeks of feeding. In contrast, female ICAM-1 -/- mice gained more weight than controls until 14 weeks on the Western-type diet (Fig. 2d). At 14 weeks, female ICAM-1 -/- mice were heavier than controls by 14.6 g; whereas in males this difference was only 7.6 g. These results indicate that the ICAM-1-dependent mechanism in regulating body weight plays a more important role in female than in male mice. At the end of the experiment, the excess white fat in males was composed mainly of subcutaneous fat (56%). In females, the major component (64%) was intra-abdominal fat (omental + retroperitoneal) resulting in prominent abdominal obesity (Fig. 3). Intra-abdominal fat is more correlated with fatty liver, hyperglycemia and higher risk of atherosclerosis than other fat depots. Such correlation may explain the increased liver weight and plasma glucose observed in females. See Table 3.

TABLE 3

Characteristics of ICAM-1 -/- mice on Western-type diet

	Body weight (g)	Body mass index (g/cm^2)	White fat-pad weight (% body weight)	Brown fat-pad weight (g)	liver weight (g)	Plasma glucose (mg/dl)
Male						
Wild type	46.24 ± 1.24	0.49 ± 0.01	17.72 ± 0.59	0.59 ± 0.04	4.75 ± 0.40	167 ± 14
ICAM-1 -/-	54.96 ± 1.64	0.56 ± 0.01	25.16 ± 1.03	0.77 ± 0.05	5.64 ± 0.38	193 ± 11
% of wild type	119% p<0.0004	114% p<0.0003	142% p<0.0001	131% p<0.006	p=0.125	p=0.178
Female						
Wild type	38.18 ± 2.11	0.44 ± 0.02	18.76 ± 1.22	0.26 ± 0.03	2.63 ± 0.23	269 ± 13
ICAM-1 -/-	56.57 ± 2.89	0.62 ± 0.02	35.48 ± 0.92	0.51 ± 0.10	3.91 ± 0.49	331 ± 21
% of wild type	148% p<0.0001	141% p<0.0001	189% p<0.0001	196% p<0.02	149% p<0.03	123% p=0.02

9 to 11 C57BL/6 mice in each group were fed a Western-type diet for 20 weeks (in male groups) or 24 weeks (in female groups) starting at 7 weeks of age. White fat-pad included the subcutaneous, inguinal, omental and retroperitoneal fat-pad. Brown fat-pad was taken from the interscapular site. Fasting plasma glucose was determined using the enzymatic assay according to manufacturer direction (Sigma Chemical Co.)

Similar to spontaneously obese ICAM-1 -/- mice, both male and female ICAM-1 -/- mice on the Western-type diet had larger brown fat depots than the controls (see Table 3). In addition, male ICAM-1 -/- mice acquired heavier hearts than the controls (0.312 ± 0.02 g vs. 0.249 ±0.02 g, $p<0.05$). Despite gaining significantly more body weight and adipose tissue mass, both male and female ICAM-1 -/- mice consumed the same amount of Western-type food as controls (approximately 40 g) during two week periods shown in Fig. 2. The obesity observed in ICAM-1 -/- mice, whether spontaneous or induced by a Western-type diet, was not due to disregulated food intake. Therefore, defects in body weight regulation observed in the ICAM-1 -/- mice was unlikely linked to changes in the production and/or function of leptin and its receptor which are known to regulate food intake in the mouse.

Example 3: Growth of wild-type and Mac-1 -/- mice on a Western-type diet (21% fat)

This example illustrates that Mac-1 -/- mice gained more weight than wild-type mice when fed a Western-type diet containing 21% fat, and thus are sensitive to diet-induced obesity.

Growth curves of wild-type and Mac-1 -/- mice on a Western-type diet were determined. See Fig. 3. 8 to 9 male mice on C57BL/6 X 129sv mixed background in each group were fed the Western-type diet starting at 9 weeks of age. A second, similar experiment was performed with identical results. From 2 to 18 weeks, $p<0.05$. Mac-1 -/- mice displayed a striking similarity in weight gain (Fig. 3) with sex-matched ICAM-1 -/- mice (Fig. 2a). After 18 weeks of feeding, the difference in body weight between Mac-1 -/- male mice and controls was 7.8 g, which was comparable to the 7.2 g difference between ICAM-1 -/- male mice and controls. Like ICAM-1 -/- mice, Mac-1 -/- mice had a significant increase in white fat (56% subcutaneous fat) and brown fat (see Table 4), while they did not consume more food than the controls (40-45 g/two week periods, shown in Fig. 4).

TABLE 4

Characteristics of Mac-1 -/- mice on Western-type diet

	Body weight (g)	Body mass index (g/cm^2)	White fat-pad weight (% body weight)	Brown fat-pad weight (g)
Wild-type	46.56 ± 1.57	0.45 ± 0.01	14.85 ± 0.58	0.43 ± 0.06
Mac-1 -/-	54.16 ± 1.66	0.51 ± 0.01	20.23 ± 1.51	0.76 ± 0.08
% of wild-type	116% p<0.006	113% p<0.009	136% p<0.004	177% p<0.004

7 to 9 male mice on C57BL/6 X 129 mixed background in each group were fed a Western-type diet for 20 weeks starting at 9 weeks of age. White fat-pad included the subcutaneous, inguinal, omental and retroperitoneal fat-pad. Brown fat-pad was taken from the interscapular site.

Example 4: Treating an individual having a body weight disorder with the gene for Mac-1 subunit αM

This example illustrates a method for treating an individual having a body weight disorder by delivering the Mac-1 subunit αM gene to hematopoietic stem cells of the individual.

Human Mac-1 subunit αM cDNA is subcloned into a restriction enzyme site of Moloney murine retrovirus so that the Mac-1 subunit αM gene is driven by the Moloney murine retrovirus long terminal repeat element. (See Walsh et al., Blood 84:453-459 (1994)). Stem cells are collected from the bone marrow of an individual having a body weight disorder. The bone marrow cells are processed on the Ceprate Stem cell Concentrator (CellPro, Inc., Bothell, WA) according to manufacturer's instructions so as to obtain a CD34-enriched population of stem cells. Transduction of the CD34-enriched stem cells is performed by culturing the CD34-enriched stem cells in fresh retroviral supernatant. The supernatant is removed and the cells are cryopreserved. The stem cells having a high level expression of Mac-1 are injected intravenously into the individual from whom the stem cells were originally collected. (See Dunbar et al., Blood 85:3048-3057 (1995)). This treatment results in alleviation of the body weight disorder.

Example 5: Treating an individual having a body weight disorder with the gene for ICAM-1

This example illustrates a method for treating an individual having a body weight disorder by delivering the ICAM-1 gene to the liver of the individual. The liver is one of the most important organs involved in lipid metabolism. A high level of expression of ICAM-1 in hepatocytes can enhance degradation of fat in the liver resulting in resistance to obesity. Liver-directed gene therapy has been successfully carried out in the treatment of hypercholesterolaemia. See Grossman et al., Nature Medicine 1:1148-1154 (1995).

An expression vector for ICAM-1 is prepared from a replication defective adenovirus (see Finkel and Epstein, FASEB J. 9:843-851 (1995)) containing an expressible cDNA copy of human ICAM-1 driven by the cytomegalovirus promotor, by cotransfection of a plasmid encoding ICAM-1 into 293 cells. Adenoviruses that are modified so as to produce less antigenicity are preferred. The viral vectors are then purified and titered. The viral vectors are delivered to the liver by portal injection or through insertion of a fine tube to the hepatic artery. Expression of ICAM-1 on the surface of the hepatocytes is verified. A fine needle aspiration of a liver sample is prepared and ICAM-1 expression is identified by a standard immunohistochemic method. This treatment results in alleviation of the body weight disorder.

Similar results are obtained if the viral vectors are delivered to adipose tissue instead of the liver.

Those skilled in the art will be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A method for determining if an animal is at risk for a body weight related disorder, comprising:

providing an animal; and

evaluating an aspect of ICAM receptor or β_2 integrin metabolism or structure in said animal, an abnormality in said aspect of ICAM receptor or β_2 integrin metabolism or structure being diagnostic of being at risk for a body weight related disorder.

2. The method of claim 1 wherein said ICAM receptor is ICAM-1.

3. The method of claim 1 wherein said β_2 integrin is Mac-1.

4. The method of claim 1 wherein said body weight related disorder is selected from the group consisting of obesity and weight loss.

5. The method of claim 4 wherein said weight loss results from a conditions selected from the group consisting of cancer, AIDS, tissue wasting, anorexia nervosa, chronic infection, gastrointestinal disease, insulin-dependent diabetes mellitus, thyrotoxicosis and malabsorption of food.

6. The method of claim 4 wherein said obesity is associated with a condition selected from the group consisting of diabetes, high blood pressure and high cholesterol levels.

7. The method of claim 1 wherein said animal is a prenatal animal.

8. The method of claim 1 wherein said abnormality in said aspect of metabolism or structure is a mutation in a gene encoding said ICAM receptor or β_2 integrin.

9. The method of claim 1 wherein said abnormality in said aspect of metabolism or structure is altered ICAM receptor or β_2 integrin polypeptide or RNA levels.

10. A method for detecting the presence of a disease affecting body weight associated

with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal, comprising:

evaluating the level of ICAM receptor or β_2 integrin polypeptide in a biological sample from a first animal; and

comparing the level obtained in the evaluating step to a level of ICAM receptor or β_2 integrin polypeptide present in a normal second animal or in said first animal at an earlier time,

wherein an increase in the level of ICAM receptor or β_2 integrin as compared to a normal level is indicative of a disease affecting body weight associated with elevated levels of ICAM receptor or β_2 integrin, and a decreased level of ICAM receptor or β_2 integrin polypeptide as compared to a normal level is indicative of a disease affecting body weight associated with decreased levels of ICAM receptor or β_2 integrin.

11. The method of claim 10 wherein said ICAM receptor is ICAM-1.

12. The method of claim 10 wherein said β_2 integrin is Mac-1.

13. The method of claim 10 wherein the evaluating step comprises:

contacting said biological sample having ICAM receptor or β_2 integrin polypeptide with an antibody that specifically binds to said ICAM receptor or β_2 integrin polypeptide under conditions which allow for formation of reaction complexes comprising said antibody and said ICAM receptor or β_2 integrin polypeptide;

detecting the formation of said reaction complexes comprising said antibody and said ICAM receptor or β_2 integrin polypeptide; and

evaluating the amount of said reaction complexes formed, wherein said amount of reaction complexes corresponds to the level of ICAM receptor or β_2 integrin polypeptide in said biological sample.

14. A method for evaluating an agent for use in modulating body weight in an animal, comprising:

providing a test cell, cell-free system or first animal having a non-wild-type pattern of ICAM receptor or β_2 integrin metabolism;

providing an agent;

administering said agent to said test cell, cell-free system or first animal in a therapeutically effective amount; and

evaluating the effect of said agent on an aspect of ICAM receptor or β_2 integrin metabolism or on a parameter related to body weight, a change in said aspect of ICAM receptor or β_2 integrin metabolism or said parameter related to body weight being indicative of the usefulness of said agent in modulating body weight in an animal.

15. The method of claim 14 wherein said agent is administered to said test cell or cell-free system in vitro, and if said change in said aspect of said ICAM receptor or β_2 integrin metabolism occurs, then further administering said agent to a test animal in a therapeutically effective amount and evaluating the in vivo effect of said agent on an aspect of ICAM receptor or β_2 integrin metabolism or on a parameter related to body weight, a change in said aspect of ICAM receptor or β_2 integrin metabolism or said parameter related to body weight being indicative of the usefulness of said agent in modulating body weight.

16. The method of claim 14 wherein said ICAM receptor is ICAM-1.

17. The method of claim 14 wherein said β_2 integrin is Mac-1.

18. The method of claim 15 wherein said second test animal has a different genotype from said test cell or cell-free system.

19. The method of claim 15 wherein said second test animal has the same genotype as said test cell or cell-free system.

20. The method of claim 14 wherein said cell is a transgenic cell and said first test animal is a non-human transgenic animal.

21. The method of claim 20 wherein said transgenic cell or transgenic non-human animal has a deletion or an addition of a gene coding for said ICAM receptor or β_2 integrin.

22. The method of claim 14 wherein said non-wild-type pattern results from overexpression of said ICAM receptor or β_2 integrin.

23. The method of claim 14 wherein said non-wild-type pattern results from underexpression of said ICAM receptor or β_2 integrin.

24. The method of claim 14 wherein said aspect of metabolism is the binding of ICAM receptor to β_2 integrin.

25. The method of claim 14 wherein said parameter related to body weight is lipid metabolism.

26. The method of claim 14 wherein said agent comprises ICAM receptor or β_2 integrin polypeptide or a biologically active fragment or analog thereof.

27. The method of claim 14 wherein said agent comprises a nucleic acid encoding ICAM receptor or β_2 integrin polypeptide or a biologically active fragment thereof.

28. The method of claim 14 wherein said agent comprises a nucleic acid encoding an ICAM receptor or β_2 integrin regulatory sequence, or a biologically active fragment thereof.

29. The method of claim 14 wherein said agent is selected from the group consisting of a binding molecule for ICAM receptor polypeptide, a binding molecule for β_2 integrin polypeptide, a binding molecule for ICAM receptor nucleic acid and a binding molecule for β_2 integrin nucleic acid.

30. The method of claim 14 wherein said agent is an antisense nucleic acid.

31. The method of claim 14 wherein said agent is selected from the group consisting of a mimetic of said ICAM receptor, a mimetic of said β_2 integrin, a mimetic of a binding molecule for said ICAM receptor, and a mimetic of a binding molecule for said β_2 integrin.

32. The method of claim 14 wherein said agent is an antibody.

33. The method of claim 14 wherein said agent is an inhibitor of a molecule that induces ICAM receptor or β_2 integrin.

34. The method of claim 14 wherein said agent is a natural ligand for ICAM receptor or β_2 integrin.

35. The method of claim 14 wherein said agent is an artificial ligand for ICAM receptor or β_2 integrin.

36. The method of claim 14 wherein said agent is selected from the group consisting of an antagonist, an agonist and a super agonist.

37. The agent identified in claim 14.

38. A method for evaluating an agent for the ability to modulate body weight in an animal, comprising:

providing an agent;

providing a compound selected from the group consisting of ICAM receptor, an extracellular portion of ICAM receptor, β_2 integrin and an extracellular portion of β_2 integrin;

contacting said agent with said ICAM receptor, extracellular portion of ICAM receptor, β_2 integrin or extracellular portion of β_2 integrin;

determining if said agent interacts with said ICAM receptor, extracellular portion of ICAM receptor, β_2 integrin or extracellular portion of β_2 integrin, and if interaction is found, then further administering said agent to a test animal in a therapeutically effective amount and evaluating the in vivo effect of said agent on said body weight of said test animal.

39. The method of claim 38 wherein said test agent is administered more than one time to said test animal.

40. The method of claim 38 wherein said interaction comprises binding of said agent with said ICAM receptor, extracellular portion of ICAM receptor, β_2 integrin or extracellular portion of β_2 integrin.

41. A method for evaluating an agent for the ability to modulate body weight in an animal by determining an alteration in the binding of ICAM receptor or β_2 integrin or extracellular portions thereof to a binding molecule, comprising:

providing an agent;

providing ICAM receptor or an extracellular portion thereof, or β_2 integrin or an extracellular portion thereof;

providing a binding molecule or an extracellular portion thereof;

combining said agent, said ICAM receptor or said extracellular portion thereof or said β_2 integrin or said extracellular portion thereof and said binding molecule or said extracellular portion thereof; and

detecting the formation of a complex comprising said ICAM receptor or said extracellular portion thereof and said binding molecule or said extracellular portion thereof, or said β_2 integrin or said extracellular portion thereof and said binding molecule or said extracellular portion thereof, an alteration in the formation of said complex in the presence of said agent as compared to in the absence of said agent being indicative of said agent altering the binding of said ICAM receptor or said extracellular portion thereof or said β_2 integrin or said extracellular portion thereof to said binding molecule; and

if said alteration in the formation of said complex occurs, then further providing a test animal and further administering said agent to said test animal in a therapeutically effective amount and evaluating the in vivo effect of said agent on said body weight of said test animal.

42. The method of claim 41 wherein altering the binding comprises inhibiting the binding.

43. The method of claim 41 wherein altering the binding comprises promoting the binding.

44. The method of claim 41 wherein said binding molecule is a β_2 integrin.

45. The method of claim 41 wherein said binding molecule is an ICAM receptor.

46. The agent identified in claim 41.

47. A method for treating a body weight related disorder in an animal, comprising:

providing an animal in need of treatment for a body weight related disorder;

providing an agent capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure;

administering said agent to said animal in a therapeutically effective amount such that treatment of said body weight related disorder occurs.

48. The method of claim 47 wherein said body weight related disorder is selected from the group consisting of obesity and weight loss.

49. The method of claim 48 wherein said weight loss results from a condition selected from the group consisting of cancer, AIDS, tissue wasting, anorexia nervosa, chronic infection, gastrointestinal disease, insulin-dependent diabetes mellitus, thyrotoxicosis and malabsorption of food.

50. The method of claim 48 wherein said obesity is associated with a condition selected from the group consisting of diabetes, high blood pressure and high cholesterol levels.

51. The method of claim 47 wherein said ICAM receptor is ICAM-1.

52. The method of claim 47 wherein said β_2 integrin is Mac-1.

53. The method of claim 47 wherein said agent comprises a nucleic acid encoding ICAM receptor or β_2 integrin polypeptide or a biologically active fragment thereof.

54. The method of claim 47 wherein said agent comprises a nucleic acid encoding an ICAM receptor or β_2 integrin regulatory sequence, or a biologically active fragment thereof.

55. The method of claim 47 wherein said agent is selected from the group consisting of a binding molecule for ICAM receptor or β_2 integrin polypeptide and a binding molecule for ICAM receptor or β_2 integrin nucleic acid.

56. The method of claim 47 wherein said agent is an antisense nucleic acid.

57. The method of claim 47 wherein said agent is selected from the group consisting of a mimetic of ICAM receptor or β_2 integrin and a mimetic of a binding molecule of ICAM receptor or β_2 integrin.

58. The method of claim 47 wherein said agent is an antibody.

59. The method of claim 47 wherein said agent is an inhibitor of a molecule that induces the expression of ICAM receptor or β_2 integrin.

60. The method of claim 47 wherein said agent is a natural ligand for ICAM receptor or β_2 integrin.

61. The method of claim 47 wherein said agent is an artificial ligand for ICAM receptor or β_2 integrin.

62. The method of claim 47 wherein said agent is selected from the group consisting of an antagonist, an agonist and a super agonist.

63. The method of claim 47 wherein said agent inhibits the interaction of ICAM receptor or β_2 integrin with a binding molecule.

64. The method of claim 47 wherein said agent inhibits the interaction between ICAM receptor and β_2 integrin.

65. The method of claim 47 wherein said agent inhibits the signal transduced by ICAM receptor or β_2 integrin.

66. A method for treating an animal at risk for a body weight related disorder, comprising:

providing an animal at risk for a body weight related disorder;

providing an agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism; and

administering said agent to said animal in a therapeutically effective amount such that treatment of said animal occurs.

67. A method for monitoring a therapeutic treatment of a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide in an animal, comprising:

evaluating the levels of ICAM receptor or β_2 integrin polypeptide in a plurality of biological samples obtained at different time points from an animal undergoing a therapeutic treatment for a disease affecting body weight associated with elevated or decreased levels of ICAM receptor or β_2 integrin polypeptide.

68. A pharmaceutical composition for treating a body weight related disorder in an animal, comprising:

a therapeutically effective amount of an agent, said agent being capable of altering an aspect of ICAM receptor or β_2 integrin metabolism or structure in said animal so as to result in treatment of said body weight related disorder in said animal; and

a pharmaceutically acceptable carrier.

69. The pharmaceutical composition of claim 68 wherein said agent is an antagonist of said ICAM receptor or β_2 integrin.

70. The pharmaceutical composition of claim 69 wherein said antagonist is selected from the group consisting of an antibody for said ICAM receptor or β_2 integrin, a fragment or analog of said ICAM receptor or β_2 integrin, a small molecule antagonist of said ICAM receptor or β_2 integrin, a mimetic of ICAM receptor or β_2 integrin, an antisense molecule for ICAM receptor or β_2 integrin and a binding molecule for ICAM receptor or β_2 integrin.

71. The pharmaceutical composition of claim 68 wherein said agent is an agonist of said ICAM receptor or β_2 integrin.

72. A method of making an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal, comprising:

providing an ICAM receptor or β_2 integrin polypeptide;

altering the amino acid sequence of said polypeptide; and

testing said altered polypeptide for an effect on an aspect of ICAM receptor or β_2 integrin metabolism, a change in said aspect of ICAM receptor or β_2 integrin metabolism being indicative of an ICAM receptor or β_2 integrin polypeptide having an antagonist or agonist activity so as to modulate body weight of an animal.

73. A method for increasing the fat content of an animal liver, comprising:

providing an animal having a liver;

administering soluble ICAM receptor or soluble β_2 integrin obtained from said animal into said animal so as to increase the fat content of said liver of said animal.

74. The method of claim 73 wherein said animal is a goose.

75. A method for increasing the fat content in milk secreted by an animal, comprising:

providing an animal capable of secreting milk;

providing an agent capable of altering an aspect of ICAM receptor or β_2 integrin structure or metabolism; and

administering said agent to said animal in an amount so as to increase the fat content in said milk of said animal.

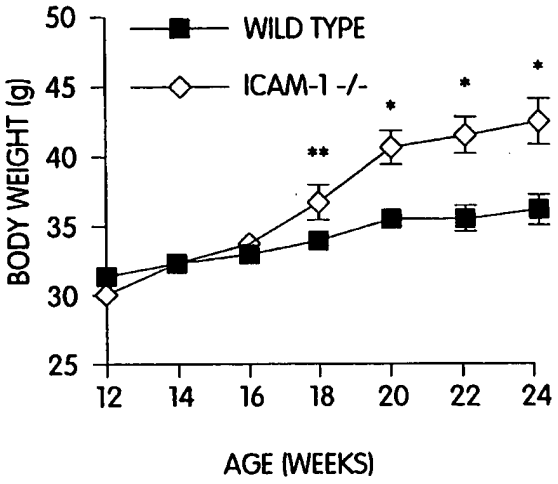
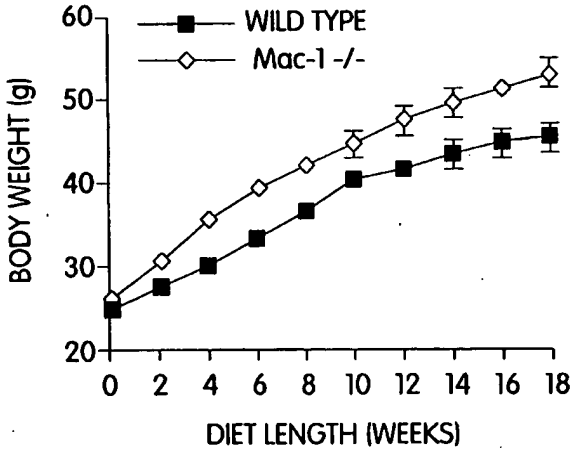

WILD TYPE
ICAM-1 -/-
BODY WEIGHT (g)
50
45
40
35
30
25
12
14
16
18
20
22
24
AGE (WEEKS)
**
*
*
*

Fig. 1


WILD TYPE
Mac-1 -/-
BODY WEIGHT (g)
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50
40
30
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0
2
4
6
8
10
12
14
16
18
DIET LENGTH (WEEKS)

Fig. 3

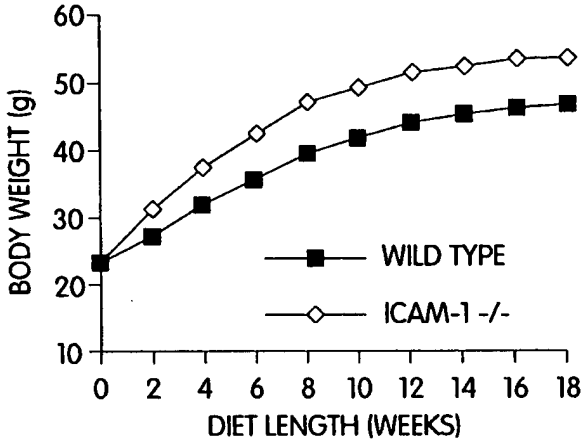

60
50
40
30
20
10
BODY WEIGHT (g)
WILD TYPE
ICAM-1 -/-
0 2 4 6 8 10 12 14 16 18
DIET LENGTH (WEEKS)

Fig. 2A

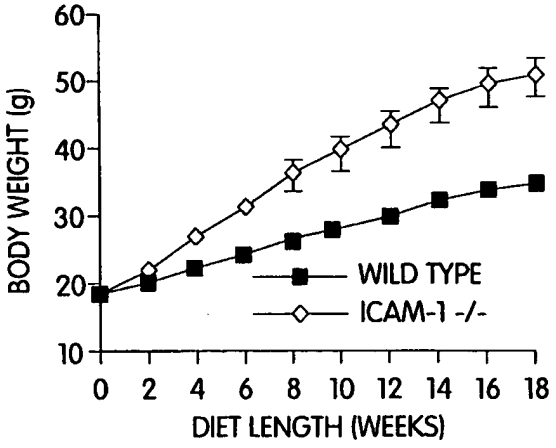
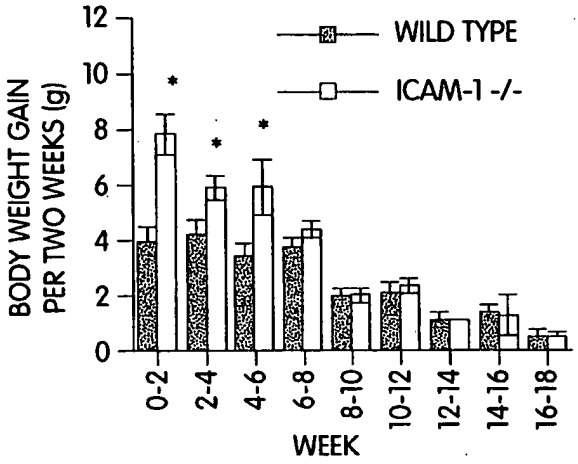

60
50
40
30
20
10
BODY WEIGHT (g)
WILD TYPE
ICAM-1 -/-
0 2 4 6 8 10 12 14 16 18
DIET LENGTH (WEEKS)

Fig. 2B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ICAM-1 -/-
BODY WEIGHT GAIN PER TWO WEEKS (g)
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10
8
6
4
2
0
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
WEEK

Fig. 2C

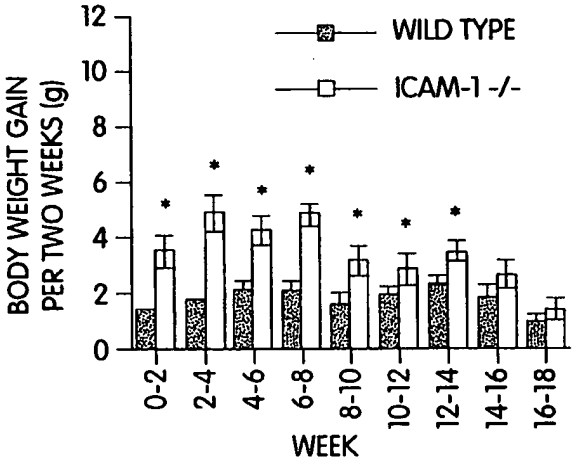
WILD TYPE
ICAM-1 -/-
BODY WEIGHT GAIN PER TWO WEEKS (g)
12
10
8
6
4
2
0
0-2
2-4
4-6
6-8
8-10
10-12
12-14
14-16
16-18
WEEK

Fig. 2D

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/00; C07K 14/705, 16/18; A61K 39/00

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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U.S. : 424/143.1, 185.1; 435/7.1, 69.3; 530/350, 387.1, 395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS: key words: weight loss, obesity, ICAM?, integrin?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PAUL, W.F. (ed), Fundamental Immunology, 3rd edition, 1993, pages 176-184, 531-541 and 1118. See entire document.	1-75
Y, P	DONG, Z. M. et al., A new class of obesity genes encodes leukocyte adhesion receptors, Proc. Nat'l Acad. Sci (USA), July, 1997, Vol. 94, pages 7526-7530, see entire document	1-75
Y	US, 5,223,396 A (ROTHLEIN ET AL.) 29 June 1993, see entire document.	1-75
Y	US 5,284,931 A, (SPRINGER ET AL.) 08 February 1994, see entire document.	1-75

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(54) Title: METHODS AND ASSOCIATED REAGENTS FOR DETECTING MODULATORS OF CYTOKINE ACTION

(57) Abstract

The present invention provides DNA constructs that contain oligonucleotide sequences comprising DNA regulatory elements of the general sequence TTN_XAA that bind activated transcriptional regulatory proteins in response to signaling molecules, such as cytokines, an operably linked promoter and operably linked heterologous gene. The present invention also provides host cells transfected with such DNA constructs, as well as methods for measuring the ability of compounds to act as agonists and antagonists of gene transcription utilizing these DNA constructs and transfected host cells.

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METHODS AND ASSOCIATED REAGENTS FOR DETECTING MODULATORS OF CYTOKINE ACTION

Field of the Invention

This invention relates to methods for detecting modulators of cytokine action, and to DNA constructs and transfected host cells useful in said assays.

Background of the Invention

In many cellular systems, extracellular signaling molecules, such as polypeptide ligands, bind to receptors on the surface of the cells, thereby triggering an intracellular signaling pathway that ultimately regulates gene transcription within the cells. For example, cytokines and growth factors, which comprise a large and diverse family of soluble polypeptides that control the growth, differentiation and function of mammalian cells, bind to specific cell surface receptors, that in some way transduce signals that elicit a specific phenotypic response. A. Miyajama et al., 10 Annu. Rev. Immunol., 295 (1992); M. Aguet et al., 55 Cell, 273 (1988); T. Kishimoto et al., 258 Science, 593 (1992) and A.Ullrich and J. Schlessinger, 61 Cell, 203 (1990). Abundant evidence shows that changes in the transcription rate of specific genes are an important component of this response. This is thought to be a consequence of alterations in the amount or the activity of specific DNA-binding proteins.

In some instances, progress has been made in defining the pathway that leads from a receptor-ligand interaction at the cell surface to changes in the activity of such DNA binding proteins or other nuclear proteins. Ulrich, 61 Cell 203. In this regard, a common response in surface receptor signaling pathways involves the activation of Ras. L.S. Mulcahy et al., 313 Nature, 241 (1985). Activated Ras then initiates a cascade of serine/threonine phosphorylations through MAP kinases leading to phosphorylation of DNA binding proteins, thereby changing their transcriptional modulatory activity. S.A. Moodie et al., 260 Science, 1658 (1993); C. A. Lange-Carter et al., 260 Science, 315 (1993); C. S. Hill et al., 73 Cell 395 (1993); H. Gille et al., 358 Nature, 414 (1992) and R. H. Chen et al., 12 Mol. Cell. Biol., 915 (1992).

Despite these advances, the signal transduction pathways utilized by many growth factors and cytokines to alter gene expression remain unclear. Thus, although known second messengers have been implicated in signal transduction in response to some of these factors, their role in modulating gene expression remains speculative. Miyajama, 10 Annu. Rev. Immunol., 295 and D.E. Levy and J.E. Darnell, 2 New Biol., 923 (1990). This in turn raises the question of how ligand specific responses are elicited in such cellular systems. Ullrich, 61 Cell, 203; M.V. Chao, 68 Cell, 995 (1992) and Levy, 2 New Biol., 923.

Progress in resolving these issues has been made recently in the interferon (IFN) system. IFNs α and β (type I) act as a primary non-specific defense against viral infections. S. Petska and J.A. Langer, 56 Annu. Rev. Biochem., 727 (1987). IFNγ (type II) has anti-viral properties but also plays a major role in regulation of the immune response. Id. Type I and type II IFNs bind to distinct cell surface receptors and cause rapid alterations in gene expression. Aguet, 55 Cell, 273; Uze, 60 Cell, 225; and G.C. Sen and P. Lengyel, 267 J. Biol. Chem., 5017 (1992). Specific sequence elements have been identified in the promoters of genes that respond to IFNα, termed interferonα stimulated response elements (ISREs), that are both necessary and sufficient for regulation by IFNα. Sen, 267 J. Biol. Chem., 5017. Specifically, activation of the IFNα receptors stimulates tyrosine phosphorylation of a family of proteins that serve as DNA binding proteins, and accordingly as transcription regulatory factors via the ISRE. C. Schindler et al., 257 Science, 809 (1992); K. Shuai et al., 258 Science, 1808 (1992) and M. J. Gutch et al., 89 Proc. Natl. Acad. Sci. USA, 11411 (1992). These DNA binding proteins, generically termed "signal transducers and activators of transcription" (STATs), assemble into a multimeric complex, translocate to the nucleus, and bind cis-acting enhancer elements in the appropriate regulatory regions. D.E. Levy et al., 3 Genes Dev., 1362 (1989); and D.S. Kessler et al., 4 Genes Dev., 1753 (1990) and Z. Zhong et al., 264 Science, 95 (1994).

One example of an IFNα-induced ISRE binding protein complex is ISGF3. T.C. Dale et al., 86 Proc. Natl. Acad. Sci., 1203 (1989) and X-Y. Fu et al., 87 Proc. Natl. Acad. Sci., 8555 (1990). ISGF3 is a complex of 4 binding proteins, called

p48, p84 (STAT1β), p91 (STAT1α) and p113 (STAT2). Recently, cDNAs encoding the proteins that constitute ISGF3 have been isolated and characterized. X-Y Fu et al., 89 Proc. Natl. Acad. Sci., 7840 (1992); C. Schindler et al., 89 Proc. Natl. Acad. Sci., 7836 (1992) and S.A. Veals et al., 12 Mol. Cell. Biol., 3315 (1992). p48 is the DNA binding component of ISGF3 and has homology to *myb*. Veals, 12 Mol. Cell. Biol., 3315. p84 and p91 are probably alternatively spliced products of the same gene and are related to p113. X-Y Fu, 89 Proc. Natl. Acad. Sci., 7840 and Schindler, 89 Proc. Natl. Acad. Sci., 7836. p84, p91 and p113 are novel proteins that contain SH2 and SH3 domains and are found in the cytoplasm of untreated cells. Schindler, 257 Science, 809 and X.Y. Fu, 70 Cell, 323-335 (1992). Thus, IFNα treatment of cells results in rapid tyrosine phosphorylation of p84, p91 and p113, causing them to associate and form a heteromeric complex with p48 to form ISGF3, which then translocates to the nucleus and binds to ISREs, stimulating transcription. Id.; Dale, 86 Proc. Natl. Acad. Sci., 1203 and Kessler, 4 Genes Dev., 1753.

Regulation in response to IFNγ is conferred by a distinct sequence from the ISRE, the gamma activated sequence (GAS). T. Decker et al., 10 EMBO J., 927 (1991); K.D. Khan et al., 90 Proc. Natl. Acad. Sci., 6806 (1993) and D.J. Lew et al., 11 Mol. Cell. Biol., 182 (1991). Treatment of cells with IFNγ results in tyrosine phosphorylation of p91 (STAT1α), which then translocates to the nucleus and binds to the GAS. Decker, 10 EMBO J,. 927 and K. Shuai et al., 258 Science, 1808 (1992). Thus the specificity of binding of either IFNα or IFNγ to their receptors is translated into a specific phosphorylation pattern within a related family of latent transcription factors (i.e. DNA binding proteins). This pattern of phosphorylation dictates the association state of the proteins, which determines specificity of binding to either an ISRE or a GAS, and the subsequent transcriptional response.

Yet another cytokine, Interleukin-6 (IL-6) plays a major role in the induction of the acute phase response in hepatocytes. The acute phase response is characterized by the dramatic transcriptional upregulation of a distinct set of genes, termed acute phase response genes. P.C. Heinrich et al, 265 Biochem. J., 621-636 (1990). Studies of the promoter regions of these genes have identified specific DNA

sequences that are required for induction of acute phase response genes by IL-6. See D.R. Kunz et al., 17 Nuc. Acids Res., 1121-1138 (1989); M. Hattori et al., 87 Proc. Natl. Acad. Sci USA, 2364-2368 (1990); K,A. Won and H. Baumann, 10 Mol. Cell. Biol., 3965-3978 (1990) and D.R. Wilson et al., 10 Mol. Cell. Biol., 6181-6191 (1990). These sequences are termed acute phase response elements (APREs). One type of APRE shows many similarities to the GAS elements that confers induction by IFNγ. Yuan et al., 14 Mol. Cell. Biol., 1657-1668 (1994). Proteins that bind to this class of APREs have been characterized and purified. U. M. Wegenka et al., 13 Mol. Cell. Biol., 276-288 (1993); T. Ito et al., 17 Nuc. Acids Res., 9425-9435 (1989) and Hattori, 87 Proc. Natl. Acad. Sci. USA, 2364-2368. A cDNA clone encoding the IL-6 - induced APRE-binding protein has been isolated (Zhong, 264 Science, 95 (1994); Akira et al, 77 Cell, 63 (1994); Zhong et al. 91 Proc. Natl. Acad. Sci., 4806 (1994) and Raz et al., 269 J. Biol. Chem., 24391 (1994)), and was found to encode a protein that shows considerable homology to p91 (STAT1α). For this reason the protein is termed STAT3. Like STAT1α, STAT3 is a latent transcription factor that is activated to bind DNA by rapid tyrosine phosphorylation.

Interleukin-4 (IL-4) is a pleiotropic cytokine that elicits biological responses in a variety of both lymphoid and non-lymphoid cell types. IL-4 is a glycoprotein of approximately 19 kD produced primarily by the Th2 subset of activated T-cells. IL-4 has since been shown to play an important role in B-cell proliferation, the regulation of immunoglobulin expression, in T-cell regulation and in the growth and differentiation of hematopoietic precursor cells. IL-4 exerts its biological effects through a specific high-affinity receptor on the surface of hematopoietic as well as certain non-hematopoietic cell lines. One chain of its receptor, the γ_c chain, is shared by the IL-2, IL-7, IL-9 and IL-13 receptors. M. Kondo et al., 262 Science 1874 (1993), M. Noguchi et al., 262 Science 1877 (1993), S. Russell et al., 262 Science 1880 (1993), and M. Kondo et al., 263 Science 1453 (1994).

Binding f IL-4 t its receptor n the cell surface results in the activation of an intracellular tyrosine kinase and the rapid phosphorylation of several proteins on tyrosine. These initial events appear to be directly related to the immediate effects f

IL-4 on target gene transcription. In particular, IL-4 up-regulates in responsive cell lines the expression of several cell-surface antigens including class II MHC, the low affinity Fc receptor for IgE (FcεRII, CD23), LFA-1 and LFA-3, CD40 and surface IgM. B. Aggarwal and J. U. Gutterman, Human Cytokines: Handbook for Basic Chemical Research Blackwell Scientific Publications, Boston, MA (1992). Perhaps the most prominent role of IL-4 is in B-cell differentiation, where IL-4 acts as a "switch factor" promoting an Ig heavy chain class switch to IgE, the major mediator of Type I allergic reactions. W.E. Paul, 77 Blood 1859 (1991). Evidence that IL-4 operates through a STAT signal transduction system is based upon the observation that IL-4 rapidly activates in a variety of cell lines phosphotyrosine-containing protein complexes that bind to a GAS-like DNA sequence element. H. Kotanides and N. Reich 262 Science 1265 (1993) and C. Schindler et al., 13 EMBO J. 1350 (1994); P. Lamb et al., 83 Blood, 2063 (1994) and I Kohler and E.P. Rieber, 23 Eur. J. Immunol., 3066 (1993). A STAT activated by IL-4 in THP-1 cells has been cloned recently (called STAT-IL-4 or STAT6) and is likely a constituent of all of the reported IL-4 induced complexes. J. Hou et al., 265 Science, 1701 (1994) and J.N. Ihle et al., 11 Trends in Genetics, 69 (1995).

Interleukin 13 (IL-13) is a pleiotropic cytokine that shares many of the biological activities of IL-4. G. Zurawski and J. E. de Vries, 15 Immunol. Today 19 (1994). IL-13 has roughly 30% sequence identity with IL-4 and exhibits IL-4-like activities on monocytes/macrophages and B-cells (A. Minty et al., 362, Nature 248 (1993) and A.N.J. McKenzie et al., 90 Proc. Natl. Acad. Sci. USA 3735 (1993). However, unlike IL-4, IL-13 has no effect on T-cells. The biological activity of IL-13 is mediated through binding to its specific high-affinity cell surface receptors consisting of an IL-13 binding subunit and one or more receptor components that are shared with the IL-4 receptor (the 'IL-4R' subunit and/or the γc subunit). G. Aversa et al., 178 J. Exp. Med. 2213 (1993). Evidence that IL-13, like IL-4, operates through a STAT signal transduction system is based upon the observation that IL-13 rapidly activates in a variety of cell lines phosphotyrosine-containing protein complexes very similar to those

induced by IL-4 that bind to a GAS-like DNA sequence element . I. Köhler et al., 345 FEBS Lett. 187 (1994).

GM-CSF belongs to a group of growth factors termed colony stimulating factors which are involved in the survival, clonal expansion, and differentiation of hematopoietic progenitor cells. J. Gasson, 77 Blood 1131 (1991) and N.A, Nicola, 58 Annu Rev. Biochem. 45 (1989). GM-CSF acts on a set of partially committed progenitor cells and causes them to divide and differentiate in the granulocyte-macrophage pathways. GM-CSF can also activate mature granulocytes and macrophages. In addition to effects on myelomonocytic lineages, GM-CSF can promote the proliferation of erythroid and megakaryocyte progenitor cells. GM-CSF, an 18-22 kD glycoprotein, is produced by a variety of cells, including T-cells, B-cells, macrophages, mast cells, endothelial cells and fibroblasts, in response to immune or inflammatory stimuli.

GM-CSF exerts its effects by interacting with cell surface receptors on specific target cells. The receptor is composed of two chains, GM-CSF-α and GM-CSF-β. L.S. Park et al., 89 Proc. Natl. Acad. Sci. 4295 (1992). The GM-CSF-α is specific to GM-CSF, while the GM-CSF-β is identical to the β subunit of the IL-5 and IL-3 receptors. G. Goodall et al., 8 Growth Factors 87 (1993). Although neither GM-CSFα or GM-CSFβ have intrinsic kinase activity, GM-CSF treatment of cells results in rapid tyrosine phosphorylation of multiple proteins. Evidence that GM-CSF operates through a STAT signal transduction system is based upon the observation that GM-CSF rapidly activates in a variety of cell lines phosphotyrosine-containing protein complexes that bind to a GAS-like DNA sequence element. A. C. Larner et al., 261 Science 1730 (1993) and P. Lamb et al., 83 Blood 2063 (1994). It has been reported that GM-CSF activates STAT5, which is likely a constituent of all of the reported GM-CSF activated complexes. Ihle et al., 11 Trends in Genetics, 69 (1995).

Interleukin-3 (IL-3) is a pleiotropic cytokine produced primarily by activated T-cells. Its effects include stimulating the proliferation and differentiation of both pluripotent hematopoietic precursor cells as well a wide variety of lineage committed cells Ihle, J.N. in Peptide Growth Factors and their Receptors Springer-

Verlag, New York (1991). The mature protein has an apparent molecular weight of 28,000, and binds to a cell surface receptor (IL-3R) that consists of at least two polypeptide chains, IL-3Rα and IL-3Rβ. The IL-3Rβ chain is also a component of the IL-5 and GM-CSF receptors, whereas the IL-3α chain is unique to the IL-3R. Miyajama et al 82 Blood 1960, (1993). Binding of IL-3 to its receptor causes the activation of the tyrosine kinase JAK2 and the rapid tyrosine phosphorylation of a set of cytoplasmic proteins. O. Silvennoinen et al., 90 Proc. Natl. Acad Sci. 8429 (1993). A GAS-binding complex that contains a member of the STAT family can be detected in extracts from cells treated with IL-3. A.C. Larner et al., 261 Science 1730 (1993); J.N. Ihle et al., 19 Trends Biochem. Sci. 222 (1994). It has been reported that IL-3 activates STAT5, which is thus likely a constituent of the reported IL-3-activated complexes. J.N. Ihle et al., 11 Trends in Genetics, 69 (1995).

Erythropoietin (Epo) is the major hormone responsible for the proliferation and maturation of red blood cell precursors. S.B. Krantz, 77 Blood 419 (1991). *In vitro* evidence indicates that it also plays a role in thrombocytopoiesis. An et al., 22 Exp. Hemat. 149 (1994). The protein, which has an apparent molecular weight of 30,000, is produced mainly in the kidneys and is induced by conditions of tissue hypoxia. It acts by binding to a cell surface receptor (EpoR) that consists of a single polypeptide chain that is a member of the hematopoietin receptor family. A. D'Andrea et al 57 Cell 277 (1989). An early event following the binding of Epo to EpoR is the activation of the tyrosine kinase JAK2, which associates non-covalently with the cytoplasmic domain of the receptor chain. B. Witthuhn et al, 74 Cell 227. Activation of JAK2 by Epo is correlated with induction of tyrosine phosphorylation of the EpoR and cytoplasmic proteins. Epo treatment of cells also results in the rapid induction of a GAS-binding activity that contains STAT proteins that are thought to contribute to Epo-induced changes in gene expression. P. Lamb et al., 83 Blood 2063 (1994); Finbloom et al., 14 Mol. Cell Biol. 2113 (1994). It has been reported that Epo activates STAT5, which is thus likely a constituent of the reported Epo-activated complexes. J.N. Ihle et al., 11 Trends in Genetics, 69 (1995).

G-CSF is a pleiotropic cytokine best known for its specific effects on the proliferation, differentiation, and activation of hematopoietic cells of the neutrophilic granulocyte lineage. G-CSF has also been reported to have chemotactic activity for human granulocytes and monocytes as well as for mesenchymal cells including fibroblasts, smooth muscle cells and myofibroblasts. These in vitro functions reflect the potential in vivo roles for G-CSF in the maintenance of steady state hematopoiesis, defense against infection, inflammation and repair. When G-CSF was administered to various animal models, an elevation of circulating neutrophils has been observed. G-CSF is now used clinically in patients that have granulopenia as a result of receiving chemotherapy or receiving immunosuppressive agents after organ transplantation. M.A.S. Moore, 9 Annu. Rev. Immunol. 159 (1991), N.A. Nicola, 58 Annu. Rev. Biochem. 45 (1989), and E. Pimentel, (1994) in Handbook of Growth Factors, Vol III, E. Pimentel, ed., CRC Press, Boca Raton, p. 177.

G-CSF exerts its biological activity through binding to G-CSFr. The receptor for G-CSF (G-CSFr) is a member of the type I cytokine receptor superfamily that lacks a kinase domain and appears to consist of a single polypeptide chain . Dimerization of two G-CSFr chains forms a high affinity binding site for G-CSF. Among the various hematopoitin receptor superfamily members, G-CSFr is most closely related to gp130, the signal-transducing component of the IL-6, oncostatin M, and leukemia inhibitory factor receptors. Recent studies have demonstrated that in myeloid leukemia cell lines, G-CSF treatment results in rapid tyrosine phosphorylation of G-CSFr , JAK1 and JAK2 tyrosine kinases and the members of the STAT family of transcription factors. S.E. Nicholson et al. 91 Proc. Natl. Acad. Sci.USA 2985 (1994) and S.S. Tian et. al., 84 Blood 1760 (1994).

It has previously been reported that many cytokines, including IL-3, GM-CSF, Epo, G-CSF, IL-4 and IL-13, activate STAT or STAT-like complexes that bind to DNA sequence elements related to the GAS elements that were first characterized in the pr moters of IFNγ-responsive genes. However, to date there has been no reported demonstrati n that the DNA sequences reported to bind to the STAT r STAT-like complexes activated by IL-3, GM-CSF, Epo, G-CSF, IL-4 and IL-13 can mediate

transcriptional induction in response to those cytokines. Accordingly, the identification of DNA sequence-elements capable of mediating transcriptional activation in response to cytokines such as IL-4, GM-CSF, G-CSF and Epo, for example, would be useful tools that would allow the responses mediated by various cytokine-activated DNA-binding proteins to be conveniently assayed.

The disclosures of the above-cited references are hereby incorporated by reference in their entirety.

Summary of the Invention

The present invention is directed to methods for screening for modulators (i.e., agonists and antagonists) of cytokine-mediated transcription, and to the DNA constructs and cytokine-responsive host cell lines transfected with such DNA constructs used in such screening methods. In a preferred embodiment, the present invention is directed to methods for screening for cytokine modulators involved in the STAT5 protein and/or STAT6 protein signaling pathway. In this regard, the DNA constructs of the present invention include oligonucleotide sequences containing regulatory elements that selectively bind activated STAT5 and/or STAT6 proteins, and modulate transcription of the associated heterologous gene, in response to appropriate signaling molecules, such as the cytokines IL-3, IL-4, IL-13, Epo, G-CSF and GM-CSF. Surprisingly, and contrary to the teaching in the art, only a limited subset of the regulatory elements that bind activated STAT5 and/or STAT6 proteins actually modulate transcription of the associated heterologous gene in the assays of the present invention.

In particular, the present invention provides a DNA construct comprising (a) an oligonucleotide sequence comprising a regulatory element of the nucleotide sequence TTN_xAA, operably linked to (b) a promoter, operably linked to (c) a heterologous gene, wherein N is independently selected from A, T, C or G and x is 4, 5, 6 or 7, and wherein the DNA construct is operably linked in such a manner that the heterologous gene is under the transcriptional control of the promoter and oligonucleotide sequence when the oligonucleotide sequence is bound by a STAT protein activated in response to IL-2, IL-3, IL-4, IL-7, IL-9, IL-13, G-CSF, GM-CSF, Epo or Tpo. Also provided is a cytokine-responsive host cell transfected with this DNA construct.

The present invention also provides a DNA construct comprising (a) an oligonucleotide sequence comprising a regulatory element of the nucleotide sequence ANTTCNNNNGAANA (SEQ ID NO. 3) operably linked to (b) a promoter, operably linked t (c) a heterol gous gene, wherein N is independently selected from A, T, C or G, and wherein the DNA construct is operably linked in such a manner that the

heterologous gene is under the transcriptional control of the promoter and oligonucleotide sequence when the oligonucleotide sequence is bound by a protein complex comprising a STAT6 protein activated in response to a cytokine. Also provided is a cytokine-responsive host cell transfected with this DNA construct.

Further, the present invention provides methods for measuring the ability of a compound to act as an agonist of gene transcription comprising (a) contacting the compound with the transfected host cells described above under conditions in which the heterologous gene is capable of being expressed in response to the compound, and (b) comparing the level of gene expression in step (a) with the level of gene expression from the host cells in the absence of the compound. Alternatively, the present invention also provides a method for measuring the ability of a compound to act as an antagonist of gene transcription comprising (a) contacting the compound with the transfected host cells described above in the presence of a predetermined amount of a cytokine under conditions in which the heterologous gene is capable of being expressed in response to the cytokine, and (b) comparing the level of gene expression in step (a) with the level of gene expression from the host cells in the presence of the cytokine, but the absence of the compound. In both these methods, the heterologous gene may be any appropriate reporter gene such as the heterologous gene for luciferase, chloramphenicol acetyl transferase, green fluorescent protein or β-galactosidase.

These and various other advantages and features of novelty which characterize the invention are pointed out with particularity in the claims annexed hereto and forming a part hereof. However, for a better understanding of the invention, its advantages, and objects obtained by its use, reference should be had to the accompanying drawings and descriptive matter, in which there is illustrated and described preferred embodiments of the invention.

Definitions

For the purposes of this invention:

"Oligonucleotide" or "DNA" molecule or sequence refers to a molecule comprised of the deoxyribonucleotides adenine (A), guanine (G), thymine (T) and/or cytosine (C), in either single-stranded form or a double-stranded helix, and comprises or includes a "regulatory element" according to the present invention, as that term is defined herein. The exact size, strandedness and orientation (i.e. 3' to 5', or 5' to 3') will depend upon many factors, which, in turn, depend upon the ultimate function and use of the oligonucleotides of the present invention. Thus, the term "oligonucleotide" or "DNA" includes double-stranded DNA found in linear DNA molecules or fragments, viruses, plasmids, vectors, chromosomes or synthetically derived DNA. As used herein, particular double-stranded DNA sequences may be described according to the normal convention of giving only the sequence in the 5' to 3' direction.

"Regulatory element" refers to a deoxyribonucleotide sequence comprising the whole, or a portion of, an oligonucleotide sequence to which an activated transcriptional regulatory protein, or a complex comprising one or more activated transcriptional regulatory proteins, binds so as to transcriptionally modulate the expression of an associated gene or genes, including heterologous genes.

"Signaling molecule" refers to an extracellular polypeptide, oligosaccaride or other non-peptidyl molecule, in either a free or bound form, that interacts with a receptor at or near the surface of a cell. This interaction in turn triggers an intracellular pathway which includes the activation of one or more transcriptional regulatory proteins that bind to a regulatory element, thereby transcriptionally modulating the expression of an associated gene or genes. As used herein, "signaling molecule" includes naturally occurring molecules, such as cytokines, peptidyl and non-peptidyl hormones, antibodies, cell-surface antigens, or synthetic mimics of any of these signaling molecules, or synthetic molecules that mimic the action of any of these signaling molecules.

"Cytokines" refer to a diverse grouping of soluble polypeptides, including growth factors and h rmones, that control the growth, differentiation and function of cells in such a manner as to ultimately elicit a phenotypic response in an rganism.

Preferred cytokines useful with the regulatory elements and associated methods of the present invention include IL-3, IL-4, IL-13, GM-CSF, G-CSF, Epo and Tpo.

"Transcriptional regulatory protein" refers to cytoplasmic or nuclear proteins that, when activated, bind the regulatory elements/oligonucleotide sequences of the present invention either directly, or indirectly through a complex of transcriptional regulatory proteins or other adapter proteins, to transcriptionally modulate the activity of an associated gene or genes. Thus, transcriptional regulatory proteins can bind directly to the DNA regulatory elements of the present invention, or can bind indirectly to the regulatory elements by binding to another protein, which in turn binds to or is bound to a DNA regulatory element of the present invention. See e.g., S.A. Veals et al., 13 Molec. Cell. Biol., 196-206 (1993). As used herein, transcriptional regulatory proteins, include, but are not limited to, those proteins referred to in the art as STAT proteins (Z. Zhong et al., 264 Science, 95) STF proteins (C. Schindler et al., 13 EMBO J., 1350 (1994)), Mammary Gland-Specific Nuclear Factor (M. Schmidt-Ney et al., 6 Mol. Endochronol., 1988 (1992) and H. Wakao et al., 267 J. Biol. Chem., 16365 (1992)), APRF (Wegenka, 13 Mol. Cell Bio., 276), GHIF (Mayer, 269 J. Biol. Chem., 4701), GHSF and EPOSF (Finbloom, 14 Mol. Cell Bio., 2113), as well as to all substantially homologous analogs and allelic variations thereof.

"Transcriptionally modulate the expression of an associated gene or genes" means to change the rate of transcription of such gene or genes.

"STAT protein" refers to those transcriptional regulatory proteins designated as "Signal Transducers and Activators of Transcription" (STAT) by Dr. J.E. Darnell of Rockefeller University. See Zhong, 264 Science 95. As used herein, STAT proteins include the p91 (STAT1), p84 (STAT1), p113 (STAT2) proteins and the STAT-associated p48 family of proteins. S.A. Veals et al., 12 Mol. Cell. Biol., 3315 (1992). Further, STAT proteins also include a binding protein designated as STAT3 (Zhong, 264 Science 95), and a binding protein designated as STAT4 (Id.). In addition, MGF is now renamed STAT5 (Gouilleux et al., 13 EMBO J., 4361-4369 (1994)) and STAT-IL-4 (or STAT6) has recently been cloned. Hou et al., 265 Science, 730 (1994)

and J.N. Ihle et al. 11 Trends in Genetics, 69 (1995). Also included are substantially homologous analogs and allelic variations of all of the above STAT proteins.

"Activate", "activated", "activation" or derivatives thereof, means that one or more transcriptional regulatory proteins within a cell are modified post-translationally, or are constituitively active, such that they can bind directly or indirectly to DNA regulatory elements/oligonucleotide sequences of the present invention in a sequence-specific manner. This modification will typically comprises phosphorylation of the transcriptional regulatory proteins via a variety of mechanisms, including, but not limited to activation by various protein kinases. See, e.g., (Shuai, 258 Science, 1808 an P. Cohen, 17 TIBS, 408 (1992)).

"DNA construct" refers to any genetic element, including, but not limited to, plasmids, vectors, chromosomes and viruses, that incorporate the oligonucleotide sequences of the present invention. For example, the DNA construct can be a vector comprising a promoter that is operably linked to an oligonucleotide sequence of the present invention, which is in turn, operably linked to a heterologous gene, such as the gene for the luciferase reporter molecule.

"Promoter" refers to a DNA regulatory region capable of binding directly or indirectly to RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of the present invention, the promoter is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter will be found a transcription initiation site (conveniently defined by mapping with S1 nuclease), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CCAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

"Gene" refers to a nucleic acid molecule, the sequence of which includes all the information required for the normal regulated production of a particular protein. A "heterologous" region of a DNA construct (i.e. a heterologous gene) is an identifiable

segment of DNA within a larger DNA construct that is not found in association with the other genetic components of the construct in nature. Thus, when the heterologous gene encodes a mammalian gene, the gene will usually be flanked by a promoter that does not flank the structural genomic DNA in the genome of the source organism.

A promoter of a DNA construct, including an oligonucleotide sequence according to the present invention, is "operably linked" to a heterologous gene when the presence of the promoter influences transcription from the heterologous gene, including genes for reporter sequences such as luciferase, chloramphenicol acetyl transferase, -galactosidase and secreted placental alkaline phosphatase. Operably linked sequences may also include two segments that are transcribed onto the same RNA transcript. Thus, two sequences, such as a promoter and a "reporter sequence" are operably linked if transcription commencing in the promoter will produce an RNA transcript of the reporter sequence. In order to be "operably linked" it is not necessary that two sequences be immediately adjacent to one another.

A host cell has been "transfected" by exogenous or heterologous DNA (e.g. a DNA construct) when such DNA has been introduced inside the cell. The transfecting DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transfecting DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stablely transfected cell is one in which the transfecting DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transfecting DNA.

"Cytokine-responsive host cell" refers to a cell line that expresses, either normally or after transfection of the requisite cDNAs, the relevant cytokine receptor components, JAK proteins, STAT proteins, and accessory factors such that, upon cytokine binding to the cell surface, STAT-mediated gene transcription is affected.

Brief Description of the Drawing

The invention may be further illustrated by reference to the accompanying Drawing wherein:

FIG. 1 is a reproduction of Electrophoretic Mobility Shift Assay (EMSA) autoradiograms that show the binding patterns of transcriptional regulatory protein-DNA binding complexes activated by IL-4 and IL-13. The EMSA's were performed as described in the Examples herein. The radiolabeled, double-stranded oligonucleotide probes utilized in the EMSAs of FIGS. 1A and 1B were made by annealing the oligonucleotides of SEQ ID NOs. 14-23 (FIG. 1A) and 24-35 (FIG. 1B).

Detailed Description of Embodiments of the Invention

The present inventors have discovered that only a select group of regulatory elements that bind activated transcriptional regulatory proteins, such as STAT proteins, actually modulate the transcription of an operably linked heterologous gene in a cell-based screen. This unexpected result is in direct contradiction to the teaching in the art that a regulatory element that binds such activated transcriptional regulatory proteins will also activate the transcription of an associated gene. Thus, the binding of a regulatory element to an activated transcriptional regulatory protein is not correlated, and provides no predictability, with respect to those regulatory elements that will activate transcription of an associated gene in response to this binding. It is only through the teaching of the present inventors that one will be able to select, without resorting to undue experimentation, regulatory elements/oligonucleotide sequences that will both bind to, and cause transactivation from, activated transcriptional regulatory proteins, such as STAT proteins.

The oligonucleotide sequences, comprising DNA regulatory elements, and that are incorporated into the DNA constructs of the present invention are selected from the nucleotide sequence TTN_xAA, wherein N is independently selected from A, T, C or G and x is 4, 5, 6 or 7. More preferably, the regulatory elements comprise oligonucleotide sequences that bind and transactivate in response to activated STAT5 and/or STAT6 proteins. These preferred oligonucleotides sequences are selected from the group consisting of TTCNNNGAA (SEQ ID NO. 1), TTCNNNNGAA (SEQ ID NO. 2) and ANTTCNNNNGAANA (SEQ. ID NO. 3), including their double stranded complements, where N is independently selected from A, T, C or G. Especially preferred oligonucleotide sequences according to the present invention include: ACTTCCCAAGAACA (SEQ ID NO. 4), ACTTCCCCGGAACA (SEQ ID NO. 5), ACTTCCCCAGAACA (SEQ ID NO. 6), ACTTCCCAGGAACA (SEQ ID NO. 7), ACTTCCTAAGAACA (SEQ ID NO. 8), ACTTCTTAAGAACA (SEQ ID NO. 9), TTCCCGGAA (SEQ ID NO. 10), TTCCCCGAA (SEQ ID NO. 11), TTCTAAGAA (SEQ ID NO. 12) and TTCTCAGAA (SEQ ID NO. 13).

The oligonucleotide sequences of the DNA constructs of the present invention can comprise the entire regulatory element alone, or can include additional flanking nucleotide sequences. In this regard, it is preferable that such oligonucleotide sequences comprise between 8 and 200 nucleotides, including the regulatory elements of the present invention. However, sequences in excess of 200 nucleotides that contain such regulatory elements, and that are capable of binding activated transcriptional regulatory proteins, and of transcriptionally modulating the expression of one or more genes thereby, are also considered to be within the scope of the present invention.

The oligonucleotide sequence component of the DNA constructs of the present invention can also comprise multimers of two or more "units" of the basic regulatory elements. In this regard, such multimer oligonucleotide sequences can, as a practical matter, contain from about 2 to 15 units of the same or different regulatory elements according to the present invention. However, theoretically, there is no limit to the number of regulatory elements within such a multimer oligonucleotide sequence. When used in the DNA construct, including a promoter and heterologous gene, according to the present invention, a multimer of the regulatory elements can enhance the expression of the gene from the DNA construct in response to various cytokines or other signaling molecules.

A variety of signaling molecules activate transcriptional regulatory proteins that bind directly or indirectly to the DNA constructs of the present invention, and modulate transcription of the operably linked heterologous gene. Nonlimiting examples of such signaling molecules include polypeptides such as cytokines and antibodies, and cell-surface antigens, oligosaccarides typically found at or near the surface of cell, non-peptidyl molecules such as TUBag4 (P. Constant et al., 264 Science, 267 (1994)) and synthetic mimics any of these molecules, in both their free and bound forms. Thus, the present invention includes cell to cell or cell to substrate transcriptional regulatory protein activation via signaling molecules bound to or near the surface of a cell or other substrate.

Preferably, the signaling molecules according t the present invention comprise cytokines that activate transcriptional regulat ry proteins, such as STAT

proteins, that bind to the regulatory elements/oligonucleotide sequences of the present invention. Examples of such cytokines include, but are not limited to, Interleukins 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13 and 15 (IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13 and IL-15), granulocyte-macrophage colony stimulating factor (GM-CSF), granuloctyte colony stimulating factor (G-CSF), colony stimulating factor 1 (CSF-1), interferons alpha, beta, and gamma (IFNα, IFNβ, IFNγ), epidermal growth factor (EGF), platelet derived growth factor (PDGF), leukemia inhibitory factor (LIF), Oncostatin M, nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), erythropoietin (Epo), thrombopoietin (Tpo), growth hormone and prolactin. Particularly preferred cytokines according to the present invention include those that activate the STAT5 protein and/or STAT6 protein pathways, including, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-7, IL-9, IL-13, IL-15, G-CSF, GM-CSF, Epo, Tpo and growth hormone.

The recombinant DNA construct, such as a reporter plasmid, according to the present invention, can be constructed using conventional molecular biology, microbiology, and recombinant DNA techniques well known to those of skill in the art. Such techniques are explained fully in the literature, including Maniatis, Fritsch & Sambrook, "Molecular Cloning: A Laboratory Manual" (1982); "DNA Cloning: A Practical Approach," Volumes I and II (D.N. Glover ed. 1985); "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells and Enzymes" [IRL Press, (1986)] and B. Perbal, "A Practical Guide to Molecular Cloning" (1984), the disclosures of which are herein incorporated by reference.

Promoter sequences useful in DNA constructs according to the present invention include all prokaryotic, eukaryotic or viral promoters capable of driving transcription of a heterologous gene of interest in combination with a regulatory element of the present invention, when transfected into an appropriate host cell. Suitable prokaryotic promoters include, but are not limited to, promoters recognized by the T4, T3, Sp6, and T7 polymerases, the P_R and P_L promoters of bacteri phage , the transcriptional regulat ry protein, recA, heat shock, and lacZ promoters of E. coli, the -

amylase and the -28-specific promoters of B. subtilis, the promoters of the bacteriophages of Bacillus, Streptomyces promoters, the int promoter of bacteriophage , the bla promoter of the β-lactamase gene of pBR322 and the CAT promoter of the chloramphenicol acetyl transferase gene of pPR325. See, e.g., B.R. Glick, 1 J. Ind. Microbiol., 277-282 (1987); Y. Cenatiempo, 68 Biochimie, 505-516 (1986); J.D. Watson et al., In: Molecular Biology of the Gene, Fourth Edition, Benjamin Cummins, Melo Park, CA (1987) and S. Gottesman, 18 Ann. Rev. Genet., 415-442 (1984), the disclosures of which are herein incorporated by reference. Preferred eukaryotic promoters include the yeast cyc-l promoter, the promoter of the mouse metallothionein I gene, the thymidine kinase promoter of the Herpes simplex virus, the SV40 early promoter, and the yeast gal-4 gene promoter. See Guarante et al., 78 Proc. Natl. Acad. Sci. USA, 2199-2203 (1981), D. Hamer et al., 1 J. Mol. Appl. Gen., 273-288 (1982), S. McKnight, 31 Cell, 355-365 (1982), C. Benoist et al., 290 Nature (London), 304-310 (1981), S.A. Johnston et al., 79 Proc Natl. Acad. Sci. (USA), 6971-6975 (1982) and P.A. Silver et al., 81 Proc. Natl. Acad. Sci. (USA), 5951-5955 (1984), the disclosures of which are herein incorporated by reference herein. Preferably, a DNA construct according to the present invention utilizes the thymidine kinase gene promoter of the Herpes simplex virus.

The third component of the recombinant DNA or construct molecules of the present invention is a "heterologous gene" which may be composed of any set of nucleotides regardless of sequence. Nonlimiting examples of such heterologous genes include the structural genes for luciferase, β-galactosidase, chloramphenicol acetyl transferase, secreted placental alkaline phosphatase, human growth hormone, tPA, green fluorescent protein and interferon. For a more extensive list of heterologous genes usable in the constructs and methods of the present invention, see Beaudet, 37 Am. J. Hum. Gen., 386-406 (1985).

Preferably the heterologous gene comprises a reporter gene whose product is used to assess regulati n of transcription via a promoter and a regulatory element/ ligonucleotide sequence of the present inventi n. The expression of this "reporter sequence" results in the formation of a reporter product (e.g., protein) which is

readily detectable. The reporter sequence will preferably be selected such that the reporter molecule will have a physical and chemical characteristics which will permit or facilitate its identification or detection by means well known in the art. In one embodiment, the presence of the reporter molecule will be detected through the use of an antibody or an antibody fragment, capable of specific binding to the reporter molecule. In another embodiment, a reporter such as β-galactosidase or luciferase can be assayed enzymatically or immunologically.

A preferred reporter molecule is LUC, well known in the art. See, e.g., J. R. De-Wet et al., 7 Mol. Cell Bio., 725 (1987). Because this is an insect gene, it is absent from mammalian cells and the enzyme product can be directly assayed in a cell extract. The level of enzyme activity corresponds to the amount of enzyme that was made, which in turn reveals the level of expression. In addition, LUC mRNA may also be measured directly.

Typically, a plasmid containing the recombinant DNA molecule of the present invention, including the LUC gene, is introduced into cytokine-responsive mammalian cells, which are then grown to, at or near confluency. In this regard, any cytokine-responsive host cell capable of activating one or more transcriptional regulatory proteins in response to an appropriate signaling molecule or molecules can be transfected with the DNA constructs of the present invention. Preferably, such cytokine-responsive host cells comprise mammalian cells, such as HepG2, U937, ME-180, TF-1 and NFS-60 cells.

The reporter cells are treated with a compound or sample suspected of containing a signaling molecule capable of inducing or activating a transcriptional regulatory protein, for example, an extract of other cytokine-treated cells. The LUC-producing reporter cells are extracted, and the soluble extracts are supplemented with luciferin and ATP. In the presence of these compounds the action of luciferase generates light, which is detected using a luminometer. The amount of light produced is directly related to the amount of luciferase present in the cellular extract.

With a suitable DNA construct f the present invention transfected into a cytokine-responsive host cell, the present invention provides a convenient means for

measuring the transcriptional activity of a reporter product in response to a signaling molecule, such as a cytokine or extract of cytokine-treated cells.

Importantly, when transcription of LUC is activated by the transcriptional regulatory protein being assayed, LUC synthesis is increased relative to a control lacking the transcriptional regulatory protein. Thus, the amount of LUC enzyme produced is an indirect measure of transcription induced by the activated transcriptional regulatory protein binding to the regulatory elements/oligonucleotide sequences of the present invention which is operably linked to the LUC gene.

When a preferred cytokine-responsive host cell, such as a HepG2 cell, is transfected with a reporter DNA construct according to the present invention, it can be utilized in assays to detect agonists and antagonists of signaling molecules that induce gene transcription via activated transcriptional regulatory proteins. As used herein, agonists or antagonists of gene transcription include compounds that intervene at any point within the signaling pathway from interaction between the signaling molecule and a cell surface receptor through activation of one or more transcriptional regulatory proteins and binding of the same to DNA regulatory elements, the end result of which is modulation of gene transcription. Further, as used herein, agonists and antagonists of gene transcription also include potentiators of known compounds with such agonist or antagonist properties. Agonists can be detected by contacting the transfected host cell with a compound or mix of compounds and, after a fixed period of time, determining the level of gene expression (e.g., the level of luciferase produced) within the treated cells. This expression level can then be compared to the expression level of the reporter gene in the absence of the compound(s). The difference between the levels of gene expression, if any, indicated whether the compound(s) of interest agonize the activation of intracellular transcriptional regulatory proteins in an analogous fashion to a known agonist signaling molecule, such as a cytokine. Further, the magnitude of the level of reporter product expressed between the treated and untreated cells provides a relative indication of the strength of that compound(s) as an agonist of gene transcription via a transcriptional regulatory protein pathway.

Alternatively, such a transfected host cell can be used to find antagonists of known agonists, e.g., cytokines such as IL-4, of gene transcription utilizing host cells transfected with the DNA constructs according to the present invention. In such an assay, the compound or compounds of interest are contacted with the host cell in conjunction with one or more known agonists (e.g., cytokines) held at a fixed concentration. The extent to which the compound(s) depress the level of gene expression in the host cell below that available from the host cell in the absence of compounds, but presence of the known agonist, provides an indication and relative strength of the antagonist properties of such compound(s).

Thus, the present invention provides methods to assay for agonists and antagonists of gene transcription utilizing the regulatory elements/oligonucleotides of the DNA constructs and transfected host cells of the present invention. Further, the agonist and antagonist compounds discovered utilizing these methods can serve as pharmaceutical agents in the intervention of various cytokine-induced disease states and conditions, or to ameliorate disease states caused by cytokine deficiency, such as inflammation, infection, anemia, cytopenia and cancerous or precancerous conditions.

The invention will be further illustrated by reference to the following non-limiting Examples.

EXAMPLE 1

Reagents

Oligonucleotides were obtained from Operon Technologies (Alameda, CA). Recombinant human GM-CSF, IL-3, IL-4 and IL-6 were obtained from R&D Systems (Minneapolis, MN). Recombinant human IL-13 was obtained from Biosource (Camarillo, CA). Recombinant human Epo and G-CSF were from Amgen, Inc. (Thousand Oaks, CA). Protease inhibitors and poly d(I-C) poly d(I-C) were from Boehringer Mannheim (Indianapolis, IN).

Cells and cell culture

U937 cells were obtained from Dr. J. E. Darnell (commercially available from ATCC) and grown in RPMI-1640 (BioWhittaker) supplemented with fetal bovine serum (10% v/v), glutamine (2 mM) and gentamicin sulfate (50 µg/mL). ME-180 cells were obtained from the ATCC and grown in McCoy's 5A (Gibco/BRL, Gaithersburg, MD) supplemented with fetal bovine serum (10% v/v), glutamine (2 mM) and gentamicin sulfate (50 µg/mL). TF-1 cells were obtained from the ATCC and grown in RPMI-1640 (BioWhittaker) supplemented with fetal bovine serum (10% v/v), glutamine (2 mM), gentamicin sulfate (50 µg/mL), and GM-CSF (5 ng/mL). IL-3-dependent NFS-60 cells were obtained from Dr. J. N. Ihle (St. Jude Children's Research Hospital, Memphis, TN) and were maintained in RPMI-1640 supplemented with fetal bovine serum (10% v/v), glutamine (2 mM) , gentamicin sulfate (50 µg/mL) and 10% WEHI-3B-conditioned medium (to provide IL-3). Factor-independent NFS-60 cells were selected by withdrawing WEHI-3B-conditioned medium from the culture medium. In about two weeks, the cells adjusted to the new growth conditions and proliferated as well as the parental NFS-60 cells. ME-180 cells were treated with cytokines at 50-75% confluency, U937, TF-1 and NFS-60 cells at a density of 2×10^5 - 1×10^6/ml. Cytokines were used at the following concentrations: IL-6, 10 ng/mL, IL-4, 10-30 ng/ml, GM-CSF, 5 ng/ml, Epo, 4-6 U/mL, IL-3, 15-20 ng/mL, IL-13, 60 ng/mL, and G-CSF, 20 ng/mL.

Preparation of nuclear extracts and Electrophoretic Mobility Shift Assays

Nuclear extracts were prepared by NP40 lysis as described in H. B. Sadowski and M. Z. Gilman, 362 Nature 79 (1993), the disclosure of which is herein incorporated by reference. Protein concentrations were measured using Bradford dye binding assay (Bio-Rad Laboratories, Hercules, CA). Nuclear extracts were prepared either from untreated U937 cells, U937 cells treated for 30 min with GM-CSF, U937 cells treated for 30 min with IL-4, TF-1 cells starved of GM-CSF for 18 h and then either left untreated r treated for 30 min with Epo, IL-3 or GM-CSF; ME-180 cells either left untreated r treated for 30 min with IL-4 or IL-13; or NFS-60 cells starved of

IL-3 for 16-18 h then either left untreated or treated for 10 min with G-CSF, IL-3 or IL-6. The double-stranded probe oligonucleotides used in the Electrophoretic Mobility Shift Assays (EMSAs) were formed by annealing oligonucleotides with the sequences:

5'-GATCCACTTCCCAAGAACAGA	-3'	(SEQ ID NO. 14)
3'- GTGAAGGGTTCTTGTCTCTAG	-5'	(SEQ ID NO. 15)
5'-GATCTGCTTCCCCGGAACGT	-3'	(SEQ ID NO. 16)
3'- ACGAAGGGGCCTTGCACTAG	-5'	(SEQ ID NO. 17)
5'-GATCTGCTTCCCCAGAACGT	-3'	(SEQ ID NO. 18)
3'- ACGAAGGGGTCTTGCACTAG	-5'	(SEQ ID NO. 19)
5'-GATCTGCTTCCCAAGAACGT	-3'	(SEQ ID NO. 20)
3'- ACGAAGGGTTCTTGCACTAG	-5'	(SEQ ID NO. 21)
5'-GATCCACTTCCCCGGAACAGA	-3'	(SEQ ID NO. 22)
3'- GTGAAGGGGCCTTGTCTCTAG	-5'	(SEQ ID NO. 23)
5'-GATCCACTTCCCCAGAACAGA	-3'	(SEQ ID NO. 24)
3'- GTGAAGGGGTCTTGTCTCTAG	-5'	(SEQ ID NO. 25)
5'-GATCCACTTCCCAGGAACAGA	-3'	(SEQ ID NO. 26)
3'- GTGAAGGGTCCTTGTCTCTAG	-5'	(SEQ ID NO. 27)
5'-GATCTACTTCCCAAGAACATA	-3'	(SEQ ID NO. 28)
3'- ATGAAGGGTTCTTGTATCTAG	-5'	(SEQ ID NO. 29)
5'-GATCCGCTTCCCAAGAACGGA	-3'	(SEQ ID NO. 30)
3'- GCGAAGGGTTCTTGCCTCTAG	-5'	(SEQ ID NO. 31)
5'-GATCCACTTCTTAAGAACAGA	-3'	(SEQ ID NO. 32)
3'- GTGAAGAATTCTTGTCTCTAG	-5'	(SEQ ID NO. 33)
5'-GATCCACTTTCCAAGAACAGA	-3'	(SEQ ID NO. 34)
3'- GTGAAAGGTTCTTGTCTCTAG	-5'	(SEQ ID NO. 35)
5'-GATCTGCTTCCCGGAACGT	-3'	(SEQ ID NO. 36)
3'- ACGAAGGGCCTTGCACTAG	-5'	(SEQ ID NO. 37)
5'-GATCGATTTCCCCGAAATG	-3'	(SEQ ID NO. 38)
3'- CTAAAGGGGCTTTACCTAG	-5'	(SEQ ID NO. 39)
5'-GATCATATTCCTGTAAGTG	-3'	(SEQ ID NO. 40)

3'-	TATAAGGACATTCACCTAG	-5'	(SEQ ID NO. 41)
5'-GATCATATTCCCGTAAGTG		-3'	(SEQ ID NO. 42)
3'-	TATAAGGGCATTCACCTAG	-5'	(SEQ ID NO. 43)
5'-GATCCATTTCTGGAAATG		-3'	(SEQ ID NO. 44)
3'-	GTAAAGACCTTTACCTAG	-5'	(SEQ ID NO. 45)
5'-GATCCATTTCCCGTAAATC		-3'	(SEQ ID NO. 46)
3'-	GTAAAGGGCATTTAGGATC	-5'	(SEQ ID NO. 47)
5'-GATCATATTACCAGAAATG		-3'	(SEQ ID NO. 48)
3'-	TATAATGGTCTTTACCTAG	-5'	(SEQ ID NO. 49)
5'-GATCATTTTCCAGTAACAG		-3'	(SEQ ID NO. 50)
3'-	TAAAAGGTCATTGTCCTAG	-5'	(SEQ ID NO. 51)
5'-GATCCAATTTCTAAGAAAGGA		-3'	(SEQ ID NO. 52)
3'-	GTTAAAGATTCTTTCCTCTAG	-5'	(SEQ ID NO. 53)
5'-GATCTGCTTCCCGAACGT		-3'	(SEQ ID NO. 54)
3'-	ACGAAGGGCTTGCACTAG	-5'	(SEQ ID NO. 55)
5'-GATCTGCTTCTCAGAACGT		-3'	(SEQ ID NO. 56)
3'-	ACGAAGAGTCTTGCACTAG	-5'	(SEQ ID NO. 57)
5'-GATCTGCTTCCCCGAACGT		-3'	(SEQ ID NO. 58)
3'-	ACGAAGGGGCTTGCACTAG	-5'	(SEQ ID NO. 59)

where the nucleotide sequences shown in bold type face correspond to nucleotide sequences, including their double-stranded complement, tested for activity as regulatory elements according to the present invention.

The annealed oligonucleotides were labeled by filling in the overhanging ends with Klenow fragment (Boehringer Mannheim) in the presence of [α-^{32}P]-dGTP and/or [α-^{32}P]-dATP (Amersham Corporation, Arlington Heights, IL). Electrophoretic mobility shift assays (EMSA's) were performed in HEPES buffer (13 mM, pH 7.6, Sigma Chemical, St. Louis, MO), containing sodium chloride (80 mM), sodium fluoride (3mM), sodium molybdate (3mM), DTT (1mM), EDTA (0.15mM), EGTA (0.15mM), glycerol (8% v/v, including contributions from the nuclear extract), poly d(I-C) poly d(I-C) (75μg/mL), radi labeled probe (approximately 0.2ng) and nuclear extract containing

5-10μg of total protein. Reactions were incubated at room temperature for 20 minutes then resolved on 5% polyacrylamide gels containing 0.25X TBE [1X TBE is Tris borate (89 mM), pH 8.0 containing EDTA (1 mM)] and glycerol (5% v/v). Gels were run at 4°C in 0.25X TBE at 20V/cm, then dried and autoradiographed.

Relative binding affinities, as determined from the EMSA results for oligonucleotide SEQ ID NOs 14-59, were visually rated and assigned according to the following scale:

(-) band corresponding to specific complex on the EMSA autoradiogram (See e.g., FIG. 1A, lane 7) barely discernible or not discernible.

(+) band corresponding to specific complex on the EMSA autoradiogram (See e.g., FIG. 1A, lanes 8 and 9) easily discernible but of weak intensity.

(++) band corresponding to specific complex on the EMSA autoradiogram (See e.g., FIG. 1A, lanes 5 and 6) easily discernible and of moderate intensity.

(+++) band corresponding to specific complex on the EMSA autoradiogram (See e.g., FIG. 1A, lanes 2 and 3) easily discernible and of strong intensity.

This visual rating system is sufficient to analyze distinguishable differences and trends in the EMSA binding data as opposed to specific numerical values. If desired, the use of a phosphor imager or densitometer (commercially available from e.g., Bio-Rad Laboratories) could provide a means to assess the differences described here quantitatively. Specific visual ratings of binding affinities for the regulatory elements of oligonucleotide SEQ ID NOs 14-41, 44-53 and 56-59 are shown in Table 1 below. Specific visual ratings of binding affinities for the regulatory elements of oligonucleotide SEQ ID NOs 36-57 are shown in Table 2 below.

Table 1: Relative EMSA binding affinities for a series of regulatory elements of double stranded configurations differing in flanking and spacing sequences to transcriptional regulatory proteins activated in response to the cytokines IL-4 and IL-13 in U-937 or ME-180 cells.

SEQ ID	Core Regulatory Element	IL-4	IL-13
14	CACTTCCCAAGAACAGA	+++	+++
16	TGCTTCCCCGGAACGT	++	++
18	TGCTTCCCCAGAACGT	+	+
20	TGCTTCCCAAGAACGT	++	++
22	CACTTCCCCGGAACAGA	++	++
24	CACTTCCCCAGAACAGA	++	++
26	CACTTCCCAGGAACAGA	++	++
28	TACTTCCCAAGAACATA	++	++
30	CGCTTCCCAAGAACGGA	++	++
32	CACTTCTTAAGAACAGA	++	++
34	CACTTTCCAAGAACAGA	++	++
36	TGCTTCCCGGAACGT	++	++
38	GATTTCCCCGAAATG	++	++
40	ATATTCCTGTAAGTG	+	n.d.
44	CATTTCTGGAAATG	++	n.d.
46	CATTTCCCGTAAATC	++	n.d.
48	ATATTACCAGAAATG	+	n.d.
50	ATTTTCCAGTAACAG	+	n.d.
52	CAATTTCTAAGAAAGGA	++	n.d.
56	TGCTTCTCAGAACGT	++	++
58	TGCTTCCCCGAACGT	++	n.d.

n.d.= not determined

Table 2: Relative EMSA binding affinities for a series of regulatory elements of double stranded configurations differing in flanking and spacing sequences to transcriptional regulatory proteins activated in response to the cytokines IL-3, GM-CSF, G-CSF.

SEQ ID No.	GM-CSF	Epo	IL-3	G-CSF (complex 1)	G-CSF (complex 2)
36	++	+	++	++	++
38	+++	+++	+++	+++	+++
40	+	+	++	n.d.	n.d.
42	+	n.d.	+	n.d.	n.d.
44	++	n.d.	+	n.d.	n.d.
46	++	+	++	++	++
48	+	+	++	n.d.	n.d.
50	+	n.d.	+	n.d.	n.d.
52	++	+++	+++	n.d.	n.d.
54	-	n.d.	-	+++	-
56	++	+	++	-	++

n.d.= not determined

The data in Table 1 show that the IL-4- and IL-13-activated STAT complexes can bind to all of the listed sequences of general structure TTN6AA with similar affinity (with the exception of SEQ ID NO. 18, which was slightly lower in affinity). The IL-4 and IL-13-activated STAT complexes can also bind to all of the listed sequences of general structure TTN5AA with varying affinities.

A specific example of the data summarized in Table 1 can be seen with respect to the EMSA autoradiograms of FIGS. 1A and 1B. In panel 1A, radiolabeled, double-stranded oligonucleotide probes made by annealing the oligonucleotides of SEQ ID NOs. 14-23 were used. Lanes marked 'UN' represent experiments using extracts from untreated cells. Other lanes are marked according to the inducing cytokine. Activated complexes can be identified by their absence in untreated extracts and their

presence in extracts treated by cytokine. The STAT complexes activated by IL-4 and IL-13 bound to all of the oligonucleotide probes with similar affinities (except SEQ ID NO. 18, which bound with a slightly lower affinity), as can be seen in Lanes 2, 3, 5, 6, 8, 9, 11, 12, 14 and 15 of panel 1A. In panel 1B, radiolabeled, double-stranded oligonucleotide probes made my annealing oligonucleotides of SEQ ID NOs. 24-35 were used. Lanes marked 'UN' represent experiments using extracts from untreated cells. Other lanes are marked according to the inducing cytokine. Activated complexes can be identified by their absence in untreated extracts and their presence in extracts treated by cytokine. The STAT complexes activated by IL-4 and IL-13 bound to all of the oligonucleotide probes with similar affinities, as can be seen in Lanes 2, 3, 5, 6, 8, 9, 11, 12, 14, 15, 17 and 18 of panel 1B.

The data in Table 2 show that the STAT complexes activated by GM-CSF, Epo, IL-3 and G-CSF can bind to a variety of DNA sequences of general structure TTN_5AA with varying affinities. In NFS-60 cells, G-CSF activated two STAT complexes that were distinguishable by their differing mobilities in an EMSA. The slower-migrating complex (Complex 1) comigrated with the STAT3 homodimer stimulated by IL-6 and was shown to contain STAT3 by antibody supershift experiments using a specific STAT3 antiserum (available from Upstate Biotechnology Incorporated, New York), and would selectively bind to a DNA sequence with a TTN_4AA structure (e.g., SEQ ID NO. 54). The faster-migrating complex (Complex 2) contained an unidentified STAT complex that migrated like the STAT complexes activated by IL-3 and GM-CSF. The two G-CSF-activated complexes had markedly different affinities for some of the regulatory elements (e.g. SEQID 54 vs SEQID 56).

Transient Transfection Assays

The reporter plasmids SEQID14x4-TK-LUC, SEQID16x4-TK-LUC, SEQID18x4-TK-LUC, SEQID20x4-TK-LUC, SEQID22x4-TK-LUC, SEQID24x4-TK-LUC, SEQID26x4-TK-LUC, SEQID28x4-TK-LUC, SEQID30x4-TK-LUC, SEQID32x4-TK-LUC, SEQID34x4-TK-LUC, SEQID36x4-TK-LUC, SEQID38x4-TK-LUC, SEQID40x4-TK-LUC, SEQID42x4-TK-LUC, SEQID44x4-TK-LUC,

SEQID46x4-TK-LUC, SEQID48x4-TK-LUC, SEQID50x4-TK-LUC, SEQID52x4-TK-LUC, SEQID52x6-TK-LUC, SEQID54x4-TK-LUC, SEQID56x4-TK-LUC, and SEQID58x4-TK-LUC contain four copies (or six copies for SEQID52x6-TK-LUC) of the same SEQ ID NOs 14-58 as those used in the EMSA's, linked to the promoter of the Herpes Simplex virus thymidine kinase gene at position -35 with respect to the cap site. See FIG. 1. The reference reporter, TK-LUC (P. Lamb et al., 83 Blood 2063 (1994)), the disclosure of which is herein incorporated by reference, is the parent vector that contains no response element. These chimeric promoters drive the expression of the structural gene for firefly luciferase.

ME-180 cells were transfected with the reporter plasmids of above by calcium phosphate coprecipitation. Cells were seeded at 1-4X10^5/ml the day before transfection. Cells were exposed to a calcium phosphate precipitate containing the above reporter plasmids (10-20 μg/ml) and the β-galactosidase-expressing plasmid pCH110 (5 μg/ml, commercially available from Pharmacia Biotech, Piscataway, NJ) for 12 h. The medium was then changed and the cells allowed to recover for 16-18 h. Recombinant cytokines were then added prediluted in growth medium and the cells harvested after 6 h. Cells were lysed and luciferase and β-galactosidase activities determined using standard techniques. See, e.g. J. R. De Wet et al., 7 Mol. Cell. Biol. 725 (1987) and Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). For each sample the normalized response was determined by dividing light units obtained from the luciferase assay with the β-galactosidase activity in the same lysate as determined using a chromogenic substrate. The results of these transfections are shown below in Table 3. Numbers given are the mean fold inductions ('fold induction' is defined as the normalized response in a cytokine-treated sample divided by the normalized response in an untreated sample). The value in parentheses is the number of independent experiments included to calculate the mean.

TF-1 cells were transfected with the reporter plasmids of above by the DEAE-dextran method as described (J. Suzow and A.D. Friedman, 13 Mol. Cell. Biol. 2141 (1993)) with the following modificati ns: test reporter constructs were added t a

concentration of 3 μg/mL during the transfection, pMSVCAT vector was not added to the transfection mixtures, the growth medium used was as described above for TF-1 cells, and cytokine inductions were carried out for 4-5 h. Cells were lysed and luciferase activity determined using standard techniques. Transfections were performed in a batch, and identical numbers of transfected cells were then separately induced with cytokine for the 4-5 h induction period. The results of these transfections are shown below in Table 4. Numbers given are the mean fold inductions ('fold induction' for TF-1 transfections is defined as the luciferase response in a cytokine-treated sample divided by the luciferase response in an untreated sample). The value in parentheses is the number of independent experiments included to calculate the mean.

NFS-60 cells were transfected with the reporter plasmids described above by the DEAE-dextran method as described in the preceding paragraph with the following modifications: test reporter constructs were added to a concentration of 6 μg/mL during the transfection, and cytokine inductions were carried out for 2.5h. The results of these transfections are shown below in Table 5.

Stable Transfection of NFS-60 Cells

Stable transfection of NFS-60 cells was accomplished as described by H. Pahl et al., 19 Exp. Hematol., 1038-1041 (1991). In brief, 1.4×10^7 factor-independent NFS-60 cells were washed twice with RPMI-1640 and then resuspended in RPMI-1640 (0.5 mL); linearized reporter DNA (16 μg) and EcoRI-digested pSV2NEO plasmid (4 μg, commercially available from ATCC) were then added. Cells and plasmids were incubated for 5 min at RT in a 0.4 cm electroporation cuvette (Bio-Rad), then subjected to a 330V, 960 μF pulse, using a Bio-Rad Gene Pulser. Cells were immediately incubated on ice for 15 min, then placed in normal growth medium (10 mL). Two days later, G418 (300 μg/mL, Boehringer-Mannheim, Indianapolis, IN) was added to the culture. Stablely transfected cells were cloned by limiting dilution. Approximately 400 clones were screened for G-CSF-inducible luciferase activity, and 16 positive clones were identified and characterized further. The results for four of the positive clones are summarized in Table 6. The number of independent experiments included to calculate the mean is indicated in parentheses.

Table 3: Transcriptional induction in ME-180 cells of reporter constructs incorporating multiple copies of test STAT regulatory elements. The values given are mean fold inductions in response to the indicated cytokine. The value in the parentheses is the number of experiments included to calculate the mean.

Reporter	Core Element	IL-4	IL-13
TK-LUC	none	0.9 (3)	1.0 (3)
SEQID14x4TK-LUC	CACTTCCCAAGAACAG	22 (3)	9.7 (3)
SEQID16x4TK-LUC	TGCTTCCCCGGAACGT	1.3 (3)	1.0 (3)
SEQID18x4TK-LUC	TGCTTCCCCAGAACGT	1.2 (3)	1.1 (3)
SEQID20x4TK-LUC	TGCTTCCCAAGAACGT	1.5 (3)	1.2 (3)
SEQID22x4TK-LUC	CACTTCCCCGGAACAG	2.7 (3)	1.6 (3)
SEQID24x4TK-LUC	CACTTCCCCAGAACAG	8.0 (3)	3.0 (3)
SEQID26x4TK-LUC	CACTTCCCAGGAACAG	10 (3)	6.4 (3)
SEQID28x4TK-LUC	TACTTCCCAAGAACAT	3.0 (3)	1.5 (3)
SEQID30x4TK-LUC	CGCTTCCCAAGAACGG	1.5 (3)	1.3 (3)
SEQID32x4TK-LUC	CACTTCTTAAGAACAG	7.3 (3)	3.3 (3)
SEQID34x4TK-LUC	CACTTTCCAAGAACAG	1.2 (3)	1.1 (3)
SEQID36x4TK-LUC	TGCTTCCCGGAACGT	1.1 (3)	n.d.
SEQID38x4TK-LUC	GATTTCCCCGAAATG	0.8 (3)	n.d.
SEQID40x4TK-LUC	ATATTCCTGTAAGTG	1.2 (3)	n.d.
SEQID44x4TK-LUC	CATTTCTGGAAATG	1.1 (3)	n.d.
SEQID46x4TK-LUC	CATTTCCCGTAAATC	1.0 (3)	n.d.
SEQID48x4TK-LUC	ATATTACCAGAAATG	1.2 (3)	n.d.
SEQID50x4TK-LUC	ATTTTCCAGTAACAG	1.0 (3)	n.d.
SEQID52x4TK-LUC	CAATTTCTAAGAAAGGA	0.8 (3)	n.d.
SEQID56x4TK-LUC	TGCTTCTCAGAACGT	1.2 (3)	n.d.
SEQID58x4TK-LUC	TGCTTCCCCGAACGT	1.2 (3)	n.d.

n.d.= not determined

Table 4: Transcriptional induction in TF-1 cells of reporter constructs incorporating multiple copies of test STAT regulatory elements. The values given are mean fold inductions in response to the indicated cytokine. The value in the parentheses is the number of experiments included to calculate the mean.

Reporter	IL-4	GM-CSF	Epo	IL-3
TK-LUC	0.7 (2)	0.8 (2)	0.8 (2)	0.7 (2)
SEQID36x4TK-LUC	n.d.	3.4 (2)	1.8 (2)	2.8 (2)
SEQID38x4TK-LUC	1.4 (2)	9.6 (4)	3.1 (4)	6.8 (3)
SEQID40x4TK-LUC	n.d.	n.d.	1.2 (2)	1.3 (2)
SEQID42x4TK-LUC	n.d.	n.d.	1.0 (2)	1.2 (2)
SEQID44x4TK-LUC	n.d.	n.d.	1.8 (3)	0.9 (3)
SEQID46x4TK-LUC	n.d.	n.d.	0.8 (2)	0.9 (2)
SEQID48x4TK-LUC	n.d.	n.d.	1.2 (2)	1.1 (2)
SEQID50x4TK-LUC	n.d.	n.d.	1.5 (2)	3.1 (2)
SEQID52x6TK-LUC	n.d.	7.6 (2)	3.5 (2)	7.3 (2)
SEQID14x4TK-LUC	3.3 (3)	1.3 (2)	0.8 (2)	1.3 (2)

n.d.= not determined

Table 5: Transcriptional induction in NFS-60 cells of reporter constructs incorporating multiple copies of test STAT regulatory elements. The values given are mean fold inductions in response to the indicated cytokine. The value in the parentheses is the number of experiments included to calculate the mean.

Reporter	Core Element	G-CSF	IL-3	IL-6
SEQID54x4TK-LUC	TTCCCGAA	14 (2)	1.0 (2)	4.1 (2)
SEQID36x4TK-LUC	TTCCCGGAA	24 (2)	3.5 (2)	4.8 (2)
SEQID56x4TK-LUC	TTCTCAGAA	3.1 (2)	1.4 (2)	1.7 (2)
SEQID38x4TK-LUC	TTCCCCGAA	24 (2)	6.1 (2)	6.9 (2)

Table 6: Transcriptional induction in NFS-60 cells stablely transfected with the SEQID38x4TK-LUC reporter plasmid. The values given are mean fold inductions in response to the indicated cytokine. The value in the parentheses is the number of experiments included to calculate the mean.

Clone Designation	G-CSF	IL-3	IL-6
1E11	17.2 (3)	16.5 (3)	3.4 (3)
6G8	17.3 (3)	20.6 (3)	3.6 (3)
1B10	16.8 (3)	27.6 (3)	2.7 (3)
4C2	12.8 (3)	20.1 (3)	2.3 (3)

The data summarized in Table 3 when compared to the in vitro binding data described above clearly demonstrate that in vitro binding is not predictive of transcriptional activity. Thus, all of the DNA elements that were incorporated as multimers into the reporter vectors bound the STAT complexes activated by IL-4 and IL-13 with a similar affinity; however, surprisingly, not all could mediate transcriptional induction in response to IL-4 and IL-13 (defined as greater than a 2-fold induction). Although it has been reported that a sequence element found in the promoter of the FcεRIIb gene (SEQID52) is necessary for the IL-4 responsiveness of this gene and can bind the STAT complex activated by IL-4 in vitro (I. Köhler et al., 345 FEBS Lett. 187 (1994)), it is clear from the data in Table 3 (SEQID52x4TK-LUC entry) that this element is not sufficient on its own to mediate IL-4 responsiveness, further underscoring the disconnection between in vitro binding data and functional, transcriptional activity.

The data summarized in Table 4 when compared to the in vitro binding data described above again clearly demonstrate that in vitro binding cannot be relied on to be predictive of transcriptional activity. Thus, many of the DNA elements that were incorporated as multimers into the reporter vectors bound the STAT complexes activated by IL-4, GM-CSF, Epo and IL-3 with varying affinities (Table 2); however,

most could not mediate a transcriptional induction in response to these cytokines (defined as greater than a 2-fold induction).

The data summarized in Table 5 again show that in vitro binding data do not reliably correlate with the ability of the elements to mediate a transcriptional induction. As described above, G-CSF activates two complexes in NFS-60 cells, one containing STAT3 and one containing an unidentified STAT protein resembling the complex activated by IL-3 in NFS-60 cells. Response elements that could bind to both G-CSF-activated complexes, such as SEQID38 and SEQID36, mediated a strong transcriptional induction in response to G-CSF, and the response element that bound strongly only to the STAT3-containing, G-CSF-activated complex (SEQID54) was activated by G-CSF (though not quite as strongly as were the sequences that bound both complexes). However, surprisingly, the response element that bound only the IL-3-activated complex (SEQID56) was not activated in response to IL-3 (defined as less than a 2-fold induction) and was only weakly activated by G-CSF.

The data summarized in Table 6 show that the NFS-60 clones stablely transfected with SEQID38x4TK-LUC respond well to both G-CSF, IL-3 and IL-6 (though the response to IL-6 was slightly lower than what was obtained in the transient transfections). Also, compared to the transiently transfected NFS-60 cells, the stable NFS-60 clones appear to respond more robustly to IL-3. Nevertheless, in general, the transient transfection data are a good indicator of what to expect when the reporter is stablely transfected into the NFS-60 cells.

It has previously been reported that many cytokines, including IL-3, GM-CSF, Epo, G-CSF, IL-4 and IL-13 activate STAT or STAT-like complexes that bind to DNA sequence elements related to the GAS element that was first characterized in the promoters of IFNγ-responsive genes. The data in Example 1 conclusively show that, surprisingly, in vitro binding is not predictive of transcriptional activation for the cytokines IL-3, IL-4, IL-13, GM-CSF, G-CSF and Epo. One can certainly not therefore assume that the in vitro STAT complex binding observed in previously published work is directly translatable into a functional reporter assay. To date there has been no reported demonstration that the DNA sequences reported t bind to the STAT or

STAT-like complexes activated by IL-3, IL-4, IL-13, GM-CSF, G-CSF or Epo can mediate transcriptional induction in response to those cytokines. Because it is not possible to extrapolate from in vitro binding data that a given sequence will be functional, the demonstration of functional activity such as that shown in the example above is absolutely critical.

While in accordance with the patent statutes, description of the preferred weight fractions, and processing conditions have been provided, the scope of the invention is not to be limited thereto or thereby. Various modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

Consequently, for an understanding of the scope of the present invention, reference is made to the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: LIGAND PHARMACEUTICALS INCORPORATED
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(C) CITY: SAN DIEGO
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(G) TELEPHONE: (619) 550-7675
(H) TELEFAX: (619) 535-3906

(ii) TITLE OF INVENTION: METHODS AND ASSOCIATED REAGENTS FOR DETECTING MODULATORS OF CYTOKINE ACTION

(iii) NUMBER OF SEQUENCES: 59

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTCNNNGAA 9

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TTCNNNNGAA 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ANTTCNNNNG AANA 14

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ACTTCCCAGG AACA 14

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ACTTCCTAAG AACA 14

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACTTCTTAAG AACA 14

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTCCCGGAA 9

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TTCCCCGAA 9

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TTCTAAGAA 9

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTCTCAGAA 9

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GATCCACTTC CCAAGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GATCTCTGTT CTTGGGAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GATCTGCTTC CCCGGAACGT 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GATCACGTTC CGGGGAAGCA 20

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GATCTGCTTC CCCAGAACGT 20

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GATCACGTTC TGGGGAAGCA 20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GATCTGCTTC CCAAGAACGT 20

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GATCACGTTC TTGGGAAGCA 20

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GATCCACTTC CCCGGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GATCTCTGTT CCGGGGAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GATCCACTTC CCCAGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GATCTCTGTT CTGGGGAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GATCCACTTC CCAGGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GATCTCTGTT CCTGGGAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GATCTACTTC CCAAGAACAT A 21

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GATCTATGTT CTTGGGAAGT A 21

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GATCCGCTTC CCAAGAACGG A 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GATCTCCGTT CTTGGGAAGC G 21

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GATCCACTTC TTAAGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GATCTCTGTT CTTAAGAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GATCCACTTT CCAAGAACAG A 21

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GATCTCTGTT CTTGGAAAGT G 21

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GATCTGCTTC CCGGAACGT 19

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GATCACGTTC CGGGAAGCA 19

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GATCGATTTC CCCGAAATG 19

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GATCCATTTC GGGGAAATC 19

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GATCATATTC CTGTAAGTG 19

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GATCCACTTA CAGGAATAT 19

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GATCATATTC CCGTAAGTG 19

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GATCCACTTA CGGGAATAT 19

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GATCCATTTC TGGAAATG 18

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GATCCATTTC CAGAAATG 18

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GATCCATTTC CCGTAAATC 19

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CTAGGATTTA CGGGAAATG 19

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GATCATATTA CCAGAAATG 19

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ther nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GATCCATTTC TGGTAATAT 19

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GATCATTTTC CAGTAACAG 19

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GATCCTGTTA CTGGAAAAT 19

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: ther nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GATCCAATTT CTAAGAAAGG A 21

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GATCTCCTTT CTTAGAAATT G 21

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GATCTGCTTC CCGAACGT 18

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GATCACGTTC GGGAAGCA 18

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

GATCTGCTTC TCAGAACGT 19

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GATCACGTTC TGAGAAGCA 19

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GATCTGCTTC CCCGAACGT 19

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "OTHER NUCLEIC ACID, SYNTHETIC DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GATCACGTTC GGGGAAGCA 19

What is claimed is:

1. A DNA construct comprising:

(a) an oligonucleotide sequence comprising a regulatory element of the nucleotide sequence TTN_xAA, operably linked to

(b) a promoter, operably linked to

(c) a heterologous gene, wherein N is independently selected from A, T, C or G and x is 4, 5, 6 or 7, and wherein the DNA construct is operably linked in such a manner that the heterologous gene is under the transcriptional control of the promoter and oligonucleotide sequence when the oligonucleotide sequence is bound by a STAT protein activated in response to IL-2, IL-3, IL-4, IL-7, IL-9, IL-13, IL-15, G-CSF, GM-CSF, Epo or Tpo.

2. A DNA construct according to claim 1, wherein the STAT protein comprises STAT5 protein and/or STAT6 protein.

3. A DNA construct according to claim 2, wherein the STAT protein is STAT6 protein.

4. A DNA construct according to claim 1, wherein the oligonucleotide sequence is selected from the group consisting of TTCNNNGAA and TTCNNNNGAA, where N is independently selected from A, T, C or G.

5. An DNA construct according to claim 4, wherein the oligonucleotide sequence is selected from the group consisting of TTCCCGGAA (SEQ ID NO. 10), TTCCCCGAA (SEQ ID NO. 11), TTCTAAGAA (SEQ ID NO. 12), TTCTCAGAA (SEQ ID NO. 13), and their double stranded complements.

6. A DNA construct according to claim 1, wherein the oligonucleotide sequence is double stranded.

7. A DNA construct according to claim 1, wherein the oligonucleotide sequence comprises a multimer of the regulatory element of claim 1.

8. A DNA construct according to claim 1, wherein the promoter is selected from the group consisting of the gene promoter of the Herpes simplex virus thymidine kinase, the adneovirus Elb and yeast alcohol dehydrogenase, and the heterologous gene is selected from the group consisting of the gene for luciferase, chloramphenicol acetyl transferase, β-galactosidase, secreted placental alkaline phosphatase, human growth hormone, t-PA, green fluorescent protein and interferon.

9. A cytokine-responsive host cell transfected with the DNA construct of claim 1.

10. A cytokine-responsive host cell according to claim 9, wherein the cell comprises a HepG2 cell, U937 cell, ME-180 cell, a TF-1 cell or NFS-60 cell.

11. A method for measuring the ability of a compound to act as an agonist of cytokine-mediated gene transcription comprising:

(a) contacting the compound with a host cell according to claim 9 under conditions in which the heterologous gene is capable of being expressed in response to the compound; and

(b) comparing the level of gene expression in step (a) with the level of gene expression from the host cell in the absence of the compound.

12. A method for measuring the ability of a compound to act as an antagonist of cytokine-mediated gene transcription comprising:

(a) contacting the compound with a host cell according to claim 9 in the presence of a predetermined amount of a cytokine under conditions in which the heterologous gene is capable of being expressed in response to the cytokine; and

(b) comparing the level of gene expression in step (a) with the level of gene expression from the host cell in the presence of the cytokine, but the absence of the compound.

13. A DNA construct comprising:

(a) an oligonucleotide sequence comprising a regulatory element of the nucleotide sequence ANTTCNNNNGAANA (SEQ ID NO. 3), or its double stranded complement, operably linked to

(b) a promoter, operably linked to

(c) a heterologous gene, wherein N is independently selected from A, T, C or G, and wherein the DNA construct is operably linked in such a manner that the heterologous gene is under the transcriptional control of the promoter and oligonucleotide sequence when the oligonucleotide sequence is bound by a STAT6 protein activated in response to a STAT6-activating cytokine.

14. A DNA construct according to claim 13, wherein the cytokine is selected from the group consisting of IL-4, IL-7, IL-9, IL-13 and IL-15.

15. A DNA construct according to claim 13, wherein the oligonucleotide sequence is selected from the group consisting of ACTTCCCAAGAACA (SEQ ID NO. 4), ACTTCCCCGGAACA (SEQ ID NO. 5), ACTTCCCCAGAACA (SEQ ID NO. 6), ACTTCCCAGGAACA (SEQ ID NO. 7), ACTTCCTAAGAACA (SEQ ID NO. 8), ACTTCTTAAGAACA (SEQ ID NO. 9), and their double stranded complements.

16. A DNA construct according to claim 13, wherein the oligonucleotide sequence is double stranded.

17. A DNA construct according to claim 13, wherein the promoter is selected from the group consisting of the gene promoter of the Herpes simplex virus thymidine kinase, the adneovirus Elb and yeast alcohol dehydrogenase, and the heterologous gene is selected from the group consisting of the gene for luciferase, chloramphenicol acetyl transferase, β-galactosidase, secreted placental alkaline phosphatase, human growth hormone, t-PA, green fluorescent protein and interferon.

18. A DNA construct according to claim 13, wherein the oligonucleotide sequence comprises a multimer of the regulatory element of claim 12.

19. A cytokine-responsive host cell transfected with the DNA construct of claim 13.

20. A cytokine-responsive host cell according to claim 19, wherein the cell comprises a HepG2 cell, U937 cell, ME-180 cell, a TF-1 cell or NFS-60 cell.

21. A method for measuring the ability of a compound to act as an agonist of cytokine-mediated gene transcription comprising:

(a) contacting the compound with a host cell according to claim 19 under conditions in which the heterologous gene is capable of being expressed in response to the compound; and

(b) comparing the level of gene expression in step (a) with the level of gene expression from the host cell in the absence of the compound.

22. A method for measuring the ability of a compound to act as an antagonist of cytokine-mediated gene transcription comprising:

(a) contacting the compound with a host cell according to claim 19 in the presence of a predetermined amount of a cytokine under conditions in which the heterologous gene is capable of being expressed in response to the cytokine; and

(b) comparing the level of gene expression in step (a) with the level of gene expression from the host cell in the presence of the cytokine, but the absence of the compound.

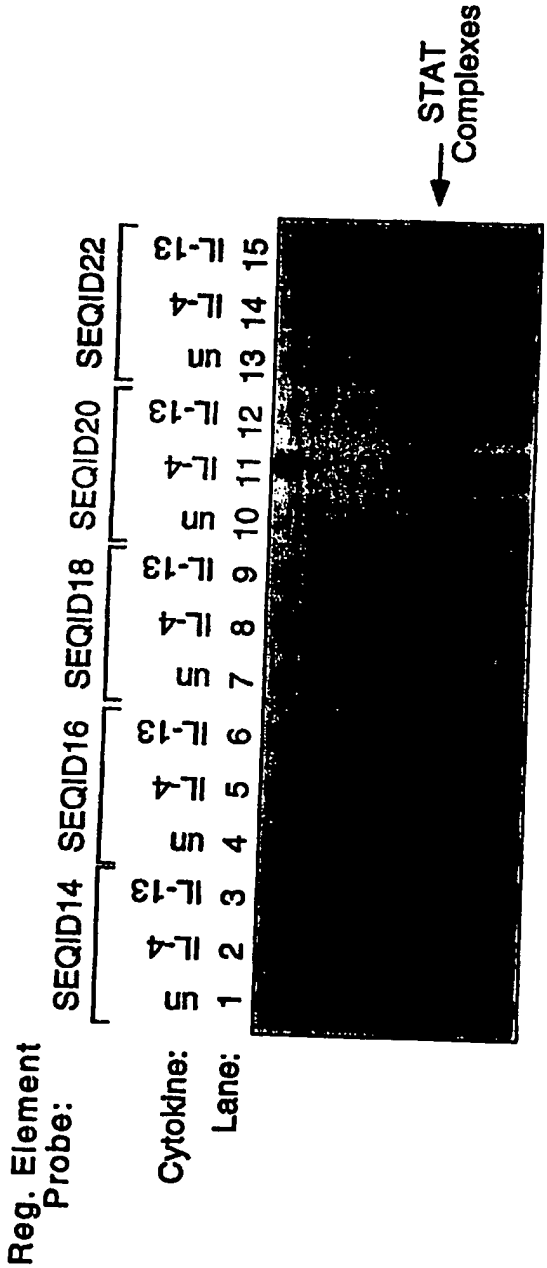
STAT Complexes
Reg. Element Probe:
SEQID14 SEQID16 SEQID18 SEQID20 SEQID22
Cytokine: un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

FIG. 1A

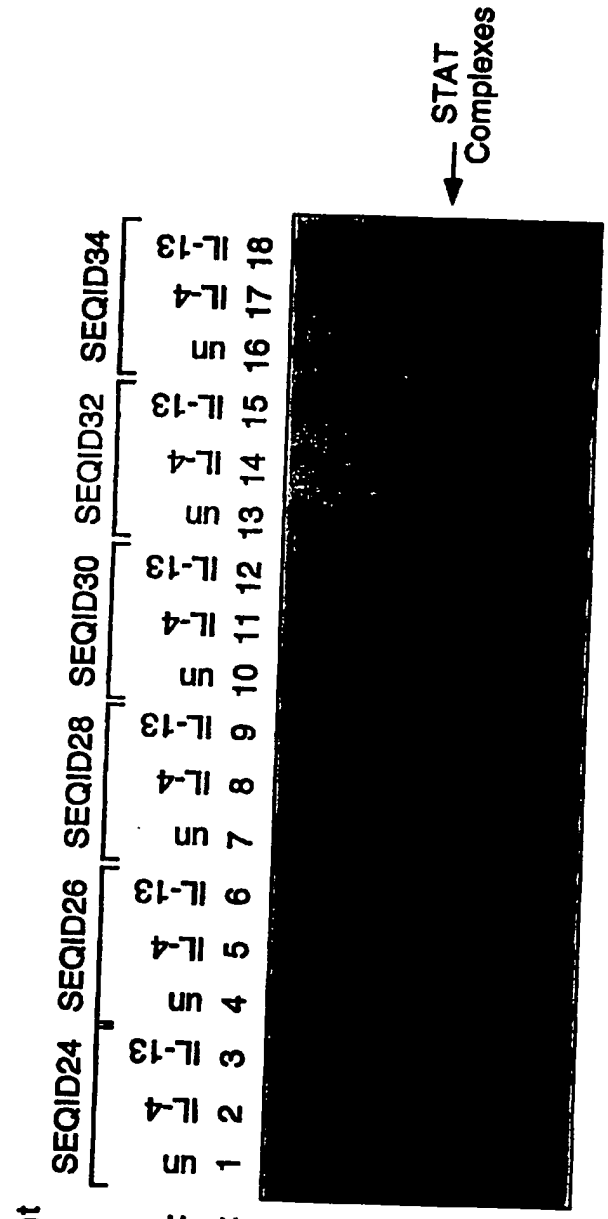
STAT Complexes
Reg. Element Probe:
SEQID24 SEQID26 SEQID28 SEQID30 SEQID32 SEQID34
Cytokine: un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13 un IL-4 IL-13
Lane: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

FIG. 1B

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 96/04012

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/85 C12N5/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 92 (7). 1995. 3041-3045. ISSN: 0027-8424, 28 March 1995, XP002009232 SEIDEL H M ET AL: "Spacing of palindromic half sites as a determinant of selective STAT (signal transducers and activators of transcription) DNA binding and transcriptional activity." see the whole document	1-10
Y	---	
Y	WO,A,95 08001 (UNIV NEW YORK) 23 March 1995 see the whole document	11,12
Y	---	
Y	EP,A,0 487 298 (SCHERING CORP) 27 May 1992 see the whole document	11,12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 1996	29.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/US 96/04012

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A	TRENDS IN BIOCHEMICAL SCIENCES, vol. 19, no. 5, May 1994, ELSEVIER, AMSTERDAM, NL, pages 222-227, XP002009234 J.N. IHLE ET AL.: "Signalling by the cytokine receptor superfamily: JAKs and STATs" cited in the application see the whole document ---	1-22
A	EMBO J., vol. 13, no. 6, June 1994, OXFORD UNIVERSITY PRESS,GB;, pages 1350-1356, XP002009235 C. SCHINDLER ET AL.: "STF-IL-4: a novel IL-4-induced signal transducing" cited in the application see the whole document ---	1-22
A	TRENDS IN GENETICS, vol. 11, no. 2, February 1995, ELSEVIER SCIENCE LTD., AMSTERDAM, NL, pages 69-74, XP002009236 J.N. IHLE AND I.M. KERR: "Jaks and Stats insignaling by the cytokine receptor superfamily" cited in the application see the whole document ---	1-10
P,X	NUCLEIC ACIDS RESEARCH 23 (16). 1995. 3283-3289. ISSN: 0305-1048, August 1995, XP002009237 LAMB P L ET AL: "STAT protein complexes activated by interferon-gamma and gp130 signaling molecules differ in their sequence preferences and transcriptional induction properties." see the whole document ---	1-22
1 P,X	WO,A,95 28482 (LIGAND PHARM INC) 26 October 1995 see the whole document --- -/--	

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Interna al Application No
PCT/US 96/04012

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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T	WO,A,95 28492 (LIGAND PHARM INC) 26 October 1995 see the whole document	1-22

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INTERNATIONAL SEARCH REPORT

Interna d Application No
PCT/US 96/04012

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WO-A-9528492	26-10-95	AU-B-	2287695	10-11-95
		AU-B-	2285995	10-11-95
		CA-A-	2165057	26-10-95
		EP-A-	0722497	24-07-96
		WO-A-	9528482	26-10-95